DIABETES

With a Chapter on HYPOGLYCEMIA

by 54 Authors

Edited by

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WITH 192 ILLUSTRATIONS, 23 IN FULL COLOR



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The discovery of hyperinsulinism does not mark an epoch in diabetes but the conditions are intimately related. In 1902, Nicholls described an island cell adenoma and in 1910 Sobolev found a hypertrophic and hyperplastic island in a diabetic at autopsy. It is possible that in the search for the internal secretion of the pancreas some investigators may have inadvertently given an excess of insulin. I have had difficulty in finding convincing evidence of this occurrence. In August, 1921, Banting and I gave 30 cc. of a potent insulin-containing extract to a diabetic dog in a deliberate and successful effort to produce hypoglycaemia. This finding was later studied in some detail in the Toronto laboratories and clinic. In 1924, Seale Harris, in extension of some previous reports, definitely suggested that spontaneous hypoglycaemia may depend on an overproduction of insulin and he coined the term "hyperinsulinism." Proof of the existence of this condition was supplied in 1927 when Wilder, Allan, Power, and Robertson of the Mayo Clinic, described a metastatic insulin tumour. The first successful removal of a pancreatic islet tumour was reported from Toronto by Campbell, Graham, and Robinson in 1939

The introduction of alloxan as a diabetogenic chemical stems from the preliminary work of Jacobs in 1937 and the production of necrosis of the beta cells of the pancreatic islets by Dunn, Sheehan, and McLetchie in 1943. In 1945, Lazarow was the first to show that glutathione could protect animals against a diabetogenic dose of alloxan. Chemicals such as cobalt affecting the α-cells are less specific than alloxan (Van Campenhout, Cornelis, and Deulin).

The work of Auguste Loubatières between 1942 and 1946 is a landmark in the history of diabetes. Following up the clinical observation of Janbon on the hypoglycaemic effects of a sulfonamide on cases of typhoid fever, Loubatières obtained experimental evidence to establish many of the principal facts we now have available to explain the mechanism of action of the oral hypoglycaemic agents. The clinical findings of Franke and Fuchs, Achelis and Hardebeck, and of Bertram, Bendfeldt, and Otto published in 1955, revived this field and set in motion the successful search for better agents and more information about their actions.

One of the most productive and promising developments in diabetes has been the emergence of many clinical investigators who are fully capable of advancing fundamental aspects of their chosen field. I could cite many names and several have been selected by our editor, who is himself a good example, to be contributors to this volume.

I am indebted to Dr. Otakar V. Sirek for a very great deal of help in preparing this article.

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PREFACE

The recent tremendous progress in many facets of research dealing with metabolic changes in diabetes and its concomitants (complications) prompted the writing of this book for scientists with varied interests, but particularly for internists, basic medical scientists, and students. It is a unique condensation of the latest and most authoritative information at both the basic science and clinical levels, written in a clear and straightforward manner by more than 40 experts from widely dispersed areas of the world. The authors were selected not only on the basis of their outstanding research contributions but also for their demonstrated effectiveness as teachers and authors. The editor made special efforts to attain coordination of the discussions throughout the book. Some repetition is unavoidable and, indeed, desirable. In attaining conciseness, we have dwelt lightly upon controversial issues and on unimportant diagnostic and therapeutic procedures. We have put much more emphasis upon new information than old. While presenting the accumulated knowledge, we have attempted to impart understanding by indicating how basic scientific observations apply to clinical problems. Many figures and tables are used for illustrations. Most chapters contain summaries of the important conclusions. A limited number of selected references are included.

Following an interesting discussion by Dr. Charles Best of major epochs in the history of diabetes, there are presented descriptions of the chemistry of insulin, its secretion, its distribution throughout the body, and its fate as well as its role in carbohydrate, fat, and protein metabolism. There also is consideration of allied actions of other hormones, particularly glucagon, epinephrine, corticosteroids, and growth hormone.

The effect of these hormones on insulin responsiveness and the stabilization of carbohydrate metabolism is stressed. Factors are described in the serum which are antagonistic to insulin, accounting for marked insulin resistance. Many aspects of the etiology, pathogenesis, pathology, clinical manifestations, and diagnosis of diabetes are discussed. There is extensive consideration of the clinical management of diabetes, including the use of general hygienic measures, diet, insulin, and oral antidiabetic agents. Meticulous attention is given to a discussion of the most recent and extensive investigations dealing with tolbutamide, chlorpropamide, metahexamide, phenethylbiguanide, and other oral antidiabetic compounds. Several chapters are devoted to major concomitants of diabetes and to different types of diabetes. The special problems of diabetes in children and during pregnancy are discussed in individual chapters. Extensive attention is given to the problem of hypoglycemia, including consideration of its many causes, damaging effects, diagnosis, and therapy.

Much of the information in this book is not only valuable in understanding the problems of diabetes but also those of obesity, arteriosclerosis, growth disturbances, adrenopathies, and other metabolic disorders.

The editor is very grateful to all of the authors and Paul B. Hoeber, Inc. for their excellent cooperation in preparing this book. There are also numerous individuals to whom all the contributors are indebted for collecting data and in preparing the manuscripts, included in this group are the editor's two secretaries, Mrs. Marguerite S. Brown and Mrs. Priscilla W. Crittenden. Eli Lilly and Company, through Dr. S. O. Waife, kindly aided in the production of the colored plates.

R. H. W.

Seattle

DIABETES

Chapter I

EPOCHS IN THE HISTORY OF DIABETES

Charles H. Best

Epochs mark the beginning and sometimes the end of an era. Certain eras, which might be termed "The Clinical Characterization of Diabetes" or "The Endocrine Glands in Diabetes," will continue indefinitely. Others, for example "The Preinsulin Era," have ended abruptly. The era of the study of "The Metabolic Lesions in Diabetes" or of "Enzymatic Abnormalities in Diabetes" will be long. Our present conception of the relative importance of eras will change and many minor and inappropriately named ones will disappear. One way of depicting eras is as coloured lines, perhaps centuries long or years short, populated at intervals by dots of various sizes representing epochs. One epoch may affect several eras and the picture which I constructed, in a preliminary way, persuaded me promptly to abandon this method of illustration!

The reference in the Papyrus Ebers to a condition that may have been diabetes mellitus is indefinite but it does suggest that the written record of our subject may go back to 1500 B.C. or even to 3000 B.C. A good clinical description was given by Celsus (30 B.C. to A.D. 50). The name "diabetes" was first introduced by another Roman physician, Aretaeus of Cappadocia (A.D. 30 to A.D. 90). The word means "to pass

through" and the disease was considered to be a weakness of the kidney because of the polyuria. It is surprising to find that in the European literature on diabetes there seems to be no mention of the presence of sugar in the urine until the seventeenth century, when Willis of Oxford noted the sweetness. He thought that this must come from the blood. Willis was by no means the first to associate glycosuria with diabetes. Avicenna, a well-known Arab physician about A.D. 1000, gave a very complete description of the disorder, including some of the complications such as diabetic gangrene, furunculosis, and phthisis and he remarked on the presence of a honeylike substance in the urine of diabetic patients. But many centuries before Avicenna, Chinese, Japanese, and Hindu writings indicate that a disorder associated with a sweet urine was known to these peoples.

The observations of Thomas Willis, however, marked the beginning of a new era when interest in diabetes quickened. Gradually it became accepted that glycosuria was a constant feature and of diagnostic value. Cawley in 1788 was apparently the first to associate diabetes with the pancreas. He found multiple calculi and destruction of pancreatic tissue at autopsy of a patient dying of diabetes. Before him, Brunner in 1682 had observed polyuria and polydypsia after removal of the pancreas in experimental animals but he did not associate this with diabetes. In 1796 a sound foundation for dietary restrictions was laid by Rollo.* In the year 1815 Chevreul identified the sugar appearing in the urine as grape sugar. In 1841 Trommer and in 1848 von Fehling developed the well-known cupric oxide tests for urinary sugar. At the same time Bouchardat outlined specific dietary schedules in which fat replaced some of the carbohydrate. He stressed the importance of low caloric intake and introduced fasting days. These principles were followed and further developed by Cantani, von Noorden, Naunyn, Allen, Falta, Petren, and many others. Bouchardat used a gluten bread. He recognized the value of (a) alcohol in moderation as food in certain cases, (b) fresh vegetables, and (c) exercise. He also used alkali in the treatment of diabetes. This had been proposed by Miahle. Fasting, undernutrition, and perhaps alkali were the only therapeutic measures that could prolong the life of a diabetic before the discovery of insulin.

The nineteenth century would have been remarkable if Claude Bernard's (1813–1878) basic contributions to our knowledge of carbohydrate metabolism had stood alone The most recent major work on Bernard (1938) written by one of my mentors in physiology, J. M. D

* Oscar Minkowski's copies of the books by Brunner and by Rollo were given to me by Frau Minkowski when Prof. B A. Houssay took me to see her in Buenos Aires in 1951.

Olmsted, presents the great experimentalist as a versatile scientific genius who was also an expert in human relationships. Claude Bernard* made several great discoveries in the field of carbohydrate metabolism. He established the presence of glycogen in the liver. He propounded the theory of sugar formation from glycogen and believed that the high blood sugar levels in diabetes were due to an overproduction of sugar by the liver. He thought of the pancreas as a gland producing only ferments for digestion of food and ascribed to it no other metabolic function. The overproduction theory propounded by Claude Bernard, almost one hundred years ago, was fostered and further developed chiefly by von Noorden and more recently in the insulin era by Soskin and Levine among others. Although Bernard did not possess a reliable blood sugar method he was able to recognize in a semiquantitative way the relationship between hyperglycaemia and glucosuria in diabetes.

Another spectacular discovery made by Bernard was that the puncture of the floor of the 4th ventricle of the brain rendered an animal temporarily diabetic.† There is still a great deal to be learned about the origin, routes, nature, and effects of the piqûre impulses.

Bernard's teachings were not always unanimously accepted and some of his own pupils, Pavy for example, disagreed with him. Pavy was the first to develop a blood sugar method that gave reliable results. He made simultaneous blood and urinary sugar determinations and went far to settle the relationship between the degree of glycosuria and the hyperglycaemia.

Von Noorden believed that both the liver and the thyroid gland are involved in diabetes. He had many revolutionary ideas and at a time when carbohydrate (as the chief offender) was omitted he introduced the oatmeal diet (1895) which considerably reduced the danger of acidosis. While the later part of the nineteenth century and the beginning of this century were marked by the personalities of von Noorden and Falta of the "Vienna School," the foremost authority on diabetes in Germany was Naunyn. He established the hereditary tendency of the disease and pointed out the difference between juvenile and aged diabetics. Naunyn was the first to introduce the term "acidosis," by which

* My copy of Bernard's "Sur le Diabète" published in 1877, which I have read many times, was presented to me by Prof. Joseph Hoet of Louvain, Belgium, in 1926 Hoet's recent work on the factors responsible for the intensification or appearance of diabetes in pregnancy and the influence of this situation on the fetus may stimulate researches which will lead to a new epoch

† My first research, started in September 1920 with E. C. Noble in J. J. R. Macleod's department, was on Bernard's piqûre. We produced the diabetes in turtles and were engaged in interrupting various nerve pathways when F. G. Banting appeared on the scene with what was to me a more promising and pressing problem

he understood accumulation of ketone bodies. Petters had identified acetone in diabetic urine in 1857 and Kussmaul found it in blood in 1874. Stadelmann, and later Minkowski and Külz, established the presence of β-oxybutyric acid in diabetic urine. Minkowski also discovered the fall in the CO_2 combining power of the blood in diabetic coma.

The early part of the twentieth century is marked by the great con- [illegible] hat [illegible] io- [illegible] ld [illegible] Dr. [illegible] of many people.

The advance of knowledge made it apparent that the "overproduction" theory was not capable of explaining many features of the diabetic state and ideas have gradually crystallized into a new concept which involves also the impaired ability of the diabetic organism to utilize carbohydrate. The extent to which each of these two processes contribute to the diabetic state and to which they are affected by insulin under physiologic conditions is not yet completely established. As knowledge of the metabolic derangements accumulated, treatment became more rational and to the names already mentioned should be added those of Lépine and Guelpa in France and of Joslin and Woodyatt on this continent. The fundamental problem, however, remained unsolved, since the cause of the disease was unknown. It was apparent that in spite of dietary restrictions of all kinds the diabetic patient, especially the juvenile, had a very unfavourable prognosis.

In 1869 Paul Langerhans discovered the islets in pancreatic tissue that were named in his honour by Laguesse. Langerhans did not appreciate the physiologic significance of the islets and considered them to be lymph glands Illness restricted his further work and he died in 1888 at age thirty [illegible] that totally depanc [illegible] and finally died in [illegible] mental proof that diabetes may be of pancreatic origin. Von Mering was not primarily interested in diabetes. He wished to study the role of the pancreas in fat digestion and it happened that Minkowski's surgical skill was available to help in his experiments. When the first depancreatized dog was passing large amounts of urine at irregular intervals, it was Minkowski who tested for sugar and rightly connected the cause of the diabetes with the removal of the pancreas. Houssay writes in his fine article on the role of Oscar Minkowski in the discovery of pancreatic diabetes.

the active principle produced by the islets might be destroyed by the acinar secretions of the pancreas, and he was responsible for the new direction taken by research on diabetes in J. J. R. Macleod's department in Toronto. Banting's surgical skill and the availability of modern laboratory procedures were combined in the investigations of 1921 and the results provided proof of the presence of the internal secretion of the pancreas.

Banting and Best in seventy-five consecutive tests in ten different depancreatized dogs, without exception, demonstrated an extensive lowering of blood and urine sugar and in many cases a dramatic clinical improvement of severely diabetic or moribund dogs. The extracts made by procedures designed to prevent the action of proteolytic enzymes, i.e., utilizing cold, acid, and alcohol, were not toxic to the dogs except when hypoglycaemia was produced. The extracts were made from degenerated dog pancreas, normal dog pancreas, foetal calf pancreas, and adult beef pancreas. The extracts of foetal calf pancreas were particularly potent and the procedure used in 1921 has subsequently provided the same clear, sterile solutions containing from 12 to 16 International Units per cubic centimeter. These have recently been given to diabetic patients. They produce sweeping falls of blood sugar and no local reactions. In the preparation of the first extracts for clinical use, Banting and Best decided to use a commercially available source—beef pancreas. These extracts had been very effective in diabetic dogs but in the amounts given to human diabetics they produced only a slight effect and elicited local reactions. The expert work of J. B. Collip further purified and concentrated these extracts from the commercially available source and this worker made other important contributions to the development of insulin. Banting and Best, while working alone, produced insulin hypoglycaemia. They noticed a decrease in ketone body excretion by qualitative tests and the absence of fatty liver in insulin-treated diabetic dogs. Their partially purified extract of whole beef pancreas kept a depancreatized dog alive for 70 days, when it was autopsied to establish the completeness of the pancreatectomy. Later with J. J. R. Macleod, J. B. Collip, and others, Banting and Best developed their preliminary observations and collaborated in new ones. The effects of insulin in restoring deposition of liver glycogen, raising the R.Q., suppressing ketonuria, producing hypoglycaemia, preventing deposition of excess fat in the liver, were established and reported in 1922. The first clinical paper on insulin was published in 1922 by Banting, Best, Collip, Campbell, and Fletcher.

Professor Macleod and new members of his department continued to add knowledge to the field of diabetes. The names of his pupils Marko-

enzyme phosphorylase and the effects of adrenaline and glucagon on its activity, the elucidation of the mechanism of formation and breakdown of glycogen and of its structure, the investigations of the mechanism of action of insulin and the emphasis on the possibility that control of the activity of glucokinase may play a key role in explaining hormonal effects in the normal and diabetic organism: these and many other advances have made this group of investigators one of the most productive of our era. Pupils and colleagues of Dr. and Mrs. Cori in turn have set up new active areas in the field of diabetes. Colowick, Krahl, Sutherland, Park, Bornstein, and others have moved on to kindle new fires.*

Experimental surgery has contributed in many important ways to our knowledge of diabetes. Dr. B. A. Houssay, who is one of the greatest of contemporary physiologists, established the hypersensitivity of hypophysectomized toads and dogs to insulin and proved for the first time that experimental diabetes produced by total extirpation of the pancreas can be ameliorated by hypophysectomy. Houssay and his colleagues also provided evidence that it is the anterior lobe of the hypophysis which is "diabetogenic" in the sense that extracts of this lobe injected into depancreatized-hypophysectomized animals aggravate the condition. Houssay and his group, Magenta, Biasotti, Rietti, Lewis, Leloir, Foglia, Martinez, Rodríguez, and others have sharply focussed the attention of experimentalists and clinicians on the importance of hormones other than insulin in the production and intensity of diabetes. In recent years, this group has contributed a great deal to our knowledge of the role of the thyroid and ovarian hormones† in the diabetic state. Houssay's work certainly marked the beginning of a great new epoch.

F. G. Young in 1937, in extension of the work of Houssay and Herbert Evans, consistently produced for the first time, permanent metahypophyseal diabetes by injecting pituitary extracts in large doses to previously normal dogs. Similar results were later obtained with the highly purified growth hormone preparations of Li and Evans. Young's work has strengthened the role of the pituitary in experimental diabetes and has stimulated a great deal of productive research on the interrelation of the anterior pituitary and pancreatic hormones.‡

* Hartroft from Toronto will help to keep those in St. Louis burning brightly!

† The work of Dwight Ingle in this field and in many others involving the continuous administration of dextrose and hormonal agents deserves special mention.

‡ I have had the benefit, over the years, of good talks with almost every contemporary authority on diabetes. Apart from my Toronto colleagues of long standing, I have had more exciting discussions on experimental diabetes with F. G. Young than with any other person with the possible exception of my older friend and one-time mentor, Sir Henry Dale. I have also seen a great deal of the fine clinicians who have been and are members of the Council of the American Diabetes

In 1920 and 1921, Fiske demonstrated a temporary decrease in th output of phosphate in the urine after ingestion of sugar. A compensator increase followed. Parallel changes occurred in blood. In 1923 Wiggles worth, Woodrow, Winter, and Smith first established that insulin de creases the inorganic phosphate of the blood. These observations mar the beginning of an epoch and are the forerunners of all the recen experiments on the effects of the diabetic state, of insulin, and othe hormones on the formation, concentration, and rate of turnover of phos phate compounds in the diabetic and normal organism. Much less i known about the changes in potassium metabolism in diabetes but a start was made by the observations of Harrop and Benedict in 1923.

The availability of insulin promptly initiated the search for the mechanism by which it acts. This is, to me, the most fascinating and fundamental feature of the problem of diabetes. It has also been frus-trating but at present is in a most fruitful phase. After the effects on blood and urinary sugar and clinical condition were established, the first experiment on the site of action was performed when Banting and Best noted the absence of any effect of a potent extract on the rate of disappearance of sugar from blood in vitro. The search for loci of action continued and direct effects on the heart, skeletal muscle, and more recently on liver have been established. The important recent contribu-tors are numerous and great advances are being made. This field has quite obviously attracted many of the finest workers in physiology and biochemistry. Levine, Stadie, Hastings, Park, Ross, Mirsky, Krahl, Wick, Chaikoff, Gemill, Drury, Stetten, Ingle, Gurin, Weinhouse, De Bodo and Steele, Fisher, Miller, Chain, Kipnis, and many to whose work I have referred specifically have elucidated the effect of insulin on the fate of dextrose, acetate, pyruvate, amino acids, and other metabolites in the intact and eviscerated organism, in isolated cardiac, diaphragmatic, and skeletal muscle, in perfused livers, in liver slices, and in other prepara-tions The work of Wertheimer and his colleagues on adipose cells in the diabetic organism and the effects of insulin have focussed attention of experimentalists and clinicians on a previously neglected but very important tissue. The same may be said for Foley's studies on the mammary gland. I must resist the temptation even to enumerate the points established but we are fortunate to have splendid reviews by Stadie, by Levine, and by Krahl in addition to those included in this volume. The effects of insulin on the nonesterified fatty acids and on mucopolysaccharides, which Dole and Dorfman will describe, may well usher in new epochs. The search for the complete explanation of the action of insulin has stimulated so many aspects of physiology and biochemistry that there may be a sense of loss when the goal is fully attained. There is no immediate cause for concern!

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Chapter 2

THE CHEMISTRY OF INSULIN

Hans Neurath and Gordon H. Dixon

ISOLATION AND HOMOGENEITY

Although the first demonstration of the importance of t pancreas in regulating blood sugar, by von Mering and Minkowski, dat back to 1890, it was about thirty-two years later that Banting and Be discovered that pancreatic extracts were active in alleviating the sym toms of hyperglycemia and glycosuria, and four years later (in 1926) J. Abel succeeded in isolating the crystalline protein from highly pu fied commercial preparations of insulin. The procedures now in use f the isolation and purification of insulin vary only in detail from t classic methods of Abel and Scott and involve extraction of fresh pa creas glands with acid, a step required in order to avoid enzymat degradation and inactivation of the hormone by the proteolytic enzym of the pancreas. While crystallization is not essential for the isolation preparations of high biologic potency, small amounts of zinc or oth metal ions, such as nickel, cobalt, or cadmium, have to be added sin any zinc initially present in the gland is removed in the course of tl acid extraction. The amount of zinc associated with crystalline insulin within the range of 0.3 to 0.6 per cent though larger amounts may l

made to combine with the protein. More recently, methods have been described whereby the protein can be crystallized from acid solutions in the absence of zinc, as the sulfate or phosphate.

The best preparations of crystalline zinc insulin appear to be homogeneous when examined by the methods of sedimentation in the ultracentrifuge or by moving boundary electrophoresis. However, when the more sensitive method of countercurrent distribution was applied (Harfenist and Craig, 1952) it was found that most crystalline samples of zinc insulin examined contained two main components in variable proportions in addition to two ill-defined minor components. The two major components were found to differ from one another in that one amide group had been removed, probably during isolation, from one of the side chains. A standard preparation of crystalline zinc insulin now available for investigative purposes through the Commission on Proteins of the International Union of Pure and Applied Chemistry represents a mixture of closely related species. A more complete review of the fractionation of insulin may be found in the publication of a recent symposium (2).

THE STRUCTURE OF INSULIN

Amino Acid Composition

The amino acid composition of insulin has been determined by several investigators, and while earlier results showed some significant discrepancies it now appears that these were due in part to experimental error and in part to differences in composition of preparations derived from various species. The most reliable data now available are summarized in Table 2-1 for insulin, derived respectively from beef, pig, and sheep (3).

Primary Structure

It was fortunate for the field of insulin chemistry that this protein was chosen by Sanger and his colleagues (9) as the first subject for the determination of a complete amino acid sequence, followed by a complete co-valent structural analysis.

The insulin monomer (MW = 6000) was shown to possess two dissimilar polypeptide chains (A and B), the N-terminus of one being glycyl- (A) and of the other, phenylalanyl- (B). These polypeptide chains, which are linked together in the native molecule by disulfide bridges, were separated by fractional precipitation at various pH's after cleavage of the disulfide bridges by oxidation with performic acid.

TABLE 2-1. AMINO ACID COMPOSITIONS OF INSULINS FROM DIFFERENT SOURCES

	Number of residues			
Amino acid	*Beef-A*	*Beef-B*	*Pork*	*Sheep*
Aspartic acid	3	3	3	3
Threonine	1	1	2	1
Serine	3	3	3	2
Glutamic acid	7	7	7	7
Proline	1	1	1	1
Glycine	4	4	4	5
Alanine	3	3	2	3
Cystine	3	3	3	3
Valine	5	5	4	5
Isoleucine	1	1	2	1
Leucine	6	6	6	6
Tyrosine	4	4	4	4
Phenylalanine	3	3	3	3
Histidine	2	2	2	2
Lysine	1	1	1	1
Arginine	1	1	1	1
Ammonia	6	5	6	6

The amino acid sequence in the phenylalanyl chain of insulin is:

phe.val.asp.glu.his.leu.cys.gly.ser.his.leu.val.glu.ala.leu.tyr.leu.val.cys.-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
gly.glu.arg.gly.phe.phe.tyr.thr.pro lys.ala.
20 21 22 23 24 25 26 27 28 29 30

In 1953, Sanger and Thompson described the structure of the second chain of insulin as follows:

gly.ileu.val.glu glu.cys.cys.ala.ser.val.cys.ser.leu.tyr.glu.leu.glu.asp.-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
tyr.cys.asp.
19 20 21

The location of the amide groups was determined by high-voltage electrophoresis of fragments obtained by partial degradation of the A and B chains with proteolytic enzymes, and these were found to be associated with glutamic acid residues 5 and 15 in the B chain and 4 in the A chain, and with aspartic acid residues 3 in the A chain and 18 in the B chain.

The next objective in the complete elucidation of the primary structure of the insulin molecule was a determination of the mode of linkage

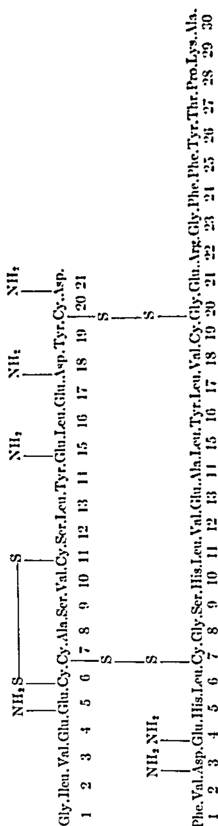
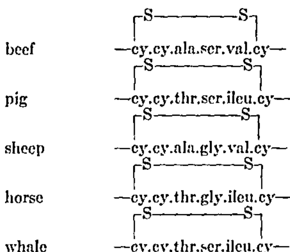


Fig. 2-1. The structure of insulin.

between the half-cystine residues in the two chains. This was accomplished by the use of proteolytic enzymes and by conditions of acid hydrolysis that would avoid disulfide interchange. An intrachain disulfide link was found between the half-cystine residues in positions 6 and 11 of the A chain, whereas the two chains were found to be linked through disulfide bonds between residue 7 of the A chain with residue 7 of the B chain and residue 19 of the A chain with residue 20 of the B chain, as shown in Figure 2-1.

Similar studies of insulin from other species showed insulin of beef, pig, sheep, hog, and whale to be identical except for some variations in residues 8, 9, and 10 in the A chain. Thus species variation seems to be reflected only and exclusively by the components of the intrachain ring in the A chain other than cystine, as shown below (1).



Three-Dimensional Structure of Insulin

Much attention has been centered in recent years upon the problem that might be termed "the higher organization" of proteins, i.e., the way in which the polypeptide chains of the protein, each possessing a fixed amino-acid sequence, fold and interact to produce the unique structure of the active protein molecule.

On the basis of much evidence, a discussion of which would be beyond the scope of this review, a characteristic mode of folding of the polypeptide backbone has been recognized in many proteins, i.e., the Pauling-Corey α-helix. In this structure the polypeptide chain is wound in a helix, the dimensions of which are such that considerable stabilization occurs by the formation of hydrogen bonds between the carboxyl and imide functions of the peptide bonds thus brought into juxtaposition. A protein possessing regions in which the polypeptide chain is folded into an α-helix is said to possess a "secondary structure." Changes in the

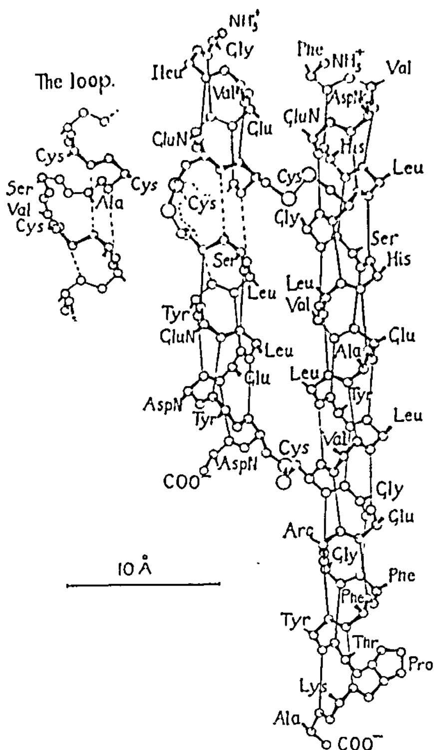


FIG. 2-2. Suggested model of insulin molecule (Linderstrøm-Lang).

secondary structure of the protein are usually accompanied by considerable changes in certain physical properties, among the most important ones being viscosity, exchangeability of hydrogen atoms for deuterium, and optical rotatory power.

The term "tertiary structure" comprises a higher degree of folding, not usually considered to be of regular form, and may entail folding of α-helices themselves and thus bring together in space certain side chains that are very far distant in the amino acid sequence. One criterion of such tertiary interactions has been spectral changes accompanying the formation or breakage of a hydrogen bond between the phenolic hydroxyl of a tyrosyl residue and a β-carboxyl or γ-carboxyl group. Potentially the most powerful tool for the determination of the three-dimensional structure is x-ray crystallography (4).

It would be beyond the scope of the present discussion to examine in detail the various configurations that have been proposed for the insulin molecule, particularly since no definitive answer to this problem is yet at hand. The presence of the disulfide bridges appears to interfere with a regular helical pattern throughout the molecular structure and for this reason Hodgkin and Oughton (2) have preferred a conformation based on "folded sheets" rather than on the α-helix. Other investigators, notably Low, Lindley and Rollett, Arndt and Riley, and Linderstrøm-Lang (6) have presented models that retain the helical configuration as the basic pattern, the latter model containing the B-chain as a left-handed α-helix, the A-chain as a right-handed one, and the intrachain disulfide loop of the A-chain being in a disorderly array. The structure proposed by Linderstrøm-Lang, though probably not correct in detail, is reproduced in Figure 2-2.

SOLUTION PROPERTIES OF INSULIN

Insulin is relatively insoluble within the pH range of 4 to 7, which includes the region of the isoelectric point (pH 5.3). Solutions of the crystalline protein appear homogeneous with respect to sedimentation in the ultracentrifuge, moving boundary electrophoresis, diffusion, and solubility (5). The molecular weight of the protein as determined by sedimentation and diffusion, osmotic pressure, or light-scattering measurements depends on the pH, ionic strength, and protein concentration since the molecule is subject to reversible aggregation. It is only under conditions minimizing intermolecular association that the molecular weight determined by physical methods agrees with the 6000 unit determined by chemical analysis. Molecular dissociation is favored by conditions of low pH and low ionic strength, by high pH (above pH

10), by concentrated solutions of guanidine hydrochloride, or by dioxane (2, 5). The insulin dimer (MW = 12,000) is the most stable unit under the conditions usually employed for physical-chemical analysis of solutions of the protein.

A characteristic property of insulin is the transformation of solutions to birefringent, thixotropic gels when heated below pH 3.5 at 100° C. This transformation, which has been studied in considerable detail by Waugh (2), involves the end-to-end aggregation of the insulin monomers into fibrils which, when viewed under the electron microscope, reveal dimensions of approximately 140 A. U. across and 10,000 A. U. in length. These fibrils are stable within the pH range of 0 to 10 and, when added as seeds to a cold solution of native insulin, will grow in length and diameter and remove all native insulin. This phenomenon can be made the basis of a method of isolation of insulin from crude extracts (8). In highly alkaline solutions (pH 12), insulin fibrils will disaggregate into monomers which by all criteria are indistinguishable from native insulin. It is believed that fibril formation results from mutual intermolecular attractions of nonpolar side chains asymmetrically disposed within the insulin molecule.

STRUCTURE AND BIOLOGIC ACTIVITY

It has been a premise of investigations of the relationship of protein structure to biologic activity that activity is due to a specific concentration of amino acid side chains (in those proteins devoid of a "prosthetic" group) upon the surface of the protein molecule. This specific concentration may be maintained by any or all of the factors known to determine protein structure, e.g. amino acid sequence, disulfide bridges, or secondary and tertiary folding. Consequently it is extremely difficult to determine whether a specific modification of the protein exerts a direct or indirect effect on biologic activity. Insulin has, perhaps, received more attention from this point of view than any other protein, but it is still not possible to say that any particular part of the insulin molecule is the active center.

While, by and large, the complete elucidation of the co-valent structure of the insulin molecule has not yet provided a clue to the relation of structure to biologic function, it is of interest to note that insulin, oxytocin, and vasopressin each contain a 20-membered disulfide ring that may have some relation to physiological function (1). It should be noted, however, that the components of this ring vary in some respect from species to species, and that the ring structure in insulin contains a half-cystine side chain that provides one of the links with the B-chain.

Many investigations have been carried out to test the essential role of side chains by reaction with specific group reagents. The literature has been well reviewed by Behrens and Bromer (1) and permits of the following conclusions: Chemical modification of most of the free amino and aliphatic hydroxyl groups appears to have no effect on biologic activity whereas esterification of the carboxyl groups or profound modification of the phenolic hydroxyl and imidazolyl groups seems to cause activity to be destroyed. Rupture of the disulfide groups by reduction or oxidation likewise causes inactivation.

Whereas enzymatic degradation of insulin by chymotrypsin, pepsin, or papain destroys hormonal activity of the protein, limited proteolysis with carboxypeptidase or trypsin produces a derivative that retains biologic activity. The sensitivity of the molecule to enzymatic degradation is indicated by the findings that removal by carboxypeptidase of the C-terminal alanine from the B-chain can be tolerated whereas removal of the other C-terminal group (asparagine) from the A-chain cannot. Removal of the C-terminal octapeptide sequence (residues 23 to 29, plus free alanine) from the B-chain by trypsin likewise causes enzyme inactivation.

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Chapter 3

CHEMISTRY OF GLUCAGON

William W. Bromer

Shortly after the discovery that pancreatic extracts alleviated the symptoms of diabetes, investigators noted that a dramatic, transient hyperglycemia preceded the customary hypoglycemic response. Kimball and Murlin suggested that the rise in blood sugar was attributable to a hyperglycemic-glycogenolytic factor called glucagon. Other workers thought that insulin might be responsible for both effects. When Abel crystallized insulin in 1926 the hyperglycemic effect was no longer observed. This provided a clear but little recognized indication that the two activities were separable. Subsequently, Scott developed a more practical method for the preparation of crystalline insulin. Scott's preparations, however, again showed the transient hyperglycemic response, and the controversy was renewed. Further evidence for the existence of glucagon came from studies showing that treatment of impure insulin with alkali or cysteine caused a marked loss of the hypoglycemic effect while the hyperglycemic activity persisted. Conversely, mild tryptic treatment of insulin preparations destroyed the hyperglycemic action without affecting the insulin action. Most of the data pointed to the presence of two hormones within the pancreas, but considerable doubt remained regarding the separate existence of a pancreatic hyperglycemic substance.

In addition to the well-known hyperglycemic-glycogenolytic activity of crude pancreas preparations, glucagon has been reported to affect (1) intestinal motility, (2) peripheral utilization of glucose, (3) lipid and protein metabolism, (4) diuresis, and (5) the production of diabetes in animals. One purpose of this chapter is to remove any doubt that glucagon is a separate chemical entity, quite distinct from insulin. The isolation of purified, crystalline glucagon, followed by chemical characterization, has provided proof for the separate existence of this substance. The isolation of glucagon was also prerequisite for an adequate exploration of its diverse metabolic effects.

ASSAY

Early progress in the isolation of glucagon was painfully slow, explainable in large measure by the lack of a precise and simple assay. Three methods of assay are currently available: (1) measurement of the rise in blood sugar in vivo, (2) stimulation of glycogenolysis and of phosphorylase in liver slices, and (3) stimulation of phosphorylase in liver homogenates. All these suffer from inherent disadvantages. The in vivo assay, while expensive, time-consuming, and not very precise ($\pm$15–25 per cent), has considerable merit because of simplicity of design and adequate sensitivity. When glucagon is injected intravenously into anesthetized cats, a dose of about 0.07 μg. per kilogram produces within 25 minutes a rise in blood sugar of approximately 30 mg. per cent. Glucagon stimulates glycogenolysis via an increase in liver phosphorylase activity (cf. Chap. 15); this effect has been used as the basis for the in vitro assays. Such methods, when compared to the in vivo assay, appear to be somewhat more precise, more sensitive, and more economical in terms of time and animals; nevertheless, they are considerably more complex in design and performance.

ISOLATION

It is difficult to isolate glucagon and insulin from the pancreas separately. Some crystalline zinc insulin preparations contain as much as 0.5 per cent glucagon. It is not surprising, then, that a crude pancreas fraction remaining after the commercial purification of zinc insulin is an appropriate starting material for the isolation of glucagon. The crude fraction contains about 4 per cent glucagon. After fractionation with cold acetone, about half of the impurities remain in the supernatant. The active precipitate is dissolved in dilute acid and is dialyzed repeatedly against dilute sodium acetate and sodium phosphate buffers. During the dialyses the hyperglycemic activity precipitates, probably in the

form of fibrils. Such glucagon preparations, which are 50 per cent to 70 per cent pure, are crystallized readily from mildly alkaline solutions.

Crystalline glucagon preparations have a high degree of purity. Repeated crystallizations afford no measurable increase in the biologic potency. Zone electrophoresis using starch as a supporting medium also provides good evidence that crystalline glucagon is a homogeneous protein. Subsequent structural analyses corroborate these data.

PROPERTIES

Glucagon is a small protein that contains no apparent prosthetic group. Traces of zinc and other metals are generally associated with

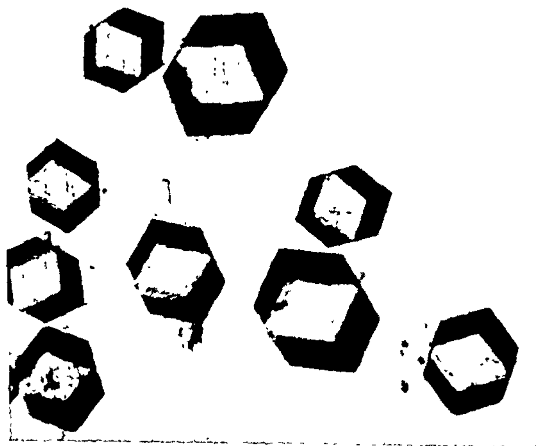


FIG. 3-1. Photomicrograph of glucagon crystals (*ca.* × 100).

glucagon crystals, but in contrast to insulin they do not form an integral or necessary part of the crystal. Glucagon is readily crystallized in the presence of a strong chelating agent such as ethylenediamine tetraacetic acid. Crystals of glucagon conform to the isometric system and appear as rhombic dodecahedra (Fig. 3-1).

Glucagon is relatively insoluble in water, particularly in the pH range from 3 to 9. On the basis of solubility and electrophoretic data, the isoelectric point of glucagon is considered to lie between pH 7.5 and 8.5. The presence of electrolytes decreases the solubility of glucagon. Mildly acidic and basic conditions increase solubility. Alkaline conditions are generally best suited for manipulation of glucagon, since acid is conducive to the aggregation of glucagon into insoluble fibrils. The alkaline stability of glucagon is commonly exploited as a means of selectively inactivating the small amounts of insulin that accompany glucagon during purification. The fibrillar aggregates of glucagon, like insulin fibrils, may be dissociated by treatment with alkali (pH 10 to 11). Present data suggest that freshly prepared glucagon fibrils, unlike insulin fibrils, retain full biologic potency.

Preliminary ultracentrifugal analyses and chemical determinations indicate that the molecular weight of glucagon is in the 3500 to 4000 range. Structural analysis provides evidence that glucagon actually has a molecular weight of 3485.

STRUCTURE

Both glucagon and insulin are hormones found in the pancreas. Both have profound effects on carbohydrate metabolism. During the course of isolation the separation of one from the other is difficult. These observations provide a basis for the reasonable assumption that, like oxytocin and vasopressin, or corticotropin and intermedin, some structural similarities may exist between the two hormones. Structural analysis of glucagon shows that the facts are quite the opposite.

Glucagon contains two free amino groups, four amide linkages, and 15 different amino acids, with a total of 29 amino acid residues. The amino acid composition of glucagon is quite different from that of insulin. Glycine, valine, histidine, lysine, alanine, tryptophan, and methionine each appear only once in the molecule. Tryptophan and methionine are not found in insulin, and cystine, isoleucine, and proline, all present in insulin, are completely absent from glucagon. Since histidine and threonine are the sole amino terminal and carboxyl terminal groups, respectively, glucagon is clearly a single, uncomplicated chain of amino acids. Insulin, on the other hand, has two chains connected by disulfide bridges. Since glucagon is completely split into its component amino acids by leucine aminopeptidase, it may be concluded that all the amino acid residues are of the L configuration and are connected by peptide bonds.

The order of arrangement of the amino acid residues in the glucagon

TABLE 3-1. STRUCTURE OF GLUCAGON

Trypsin (2.25 hr.)	His(ser,glu,gly,thr,phe,thr,ser,asp,tyr,ser,lys) tyr(leu,asp,ser,arg) arg(ala,glu,asp,phe,val,glu,try,leu,met,asp,thr) arg ala(glu,asp,phe,val,glu,try,leu,met,asp,thr)
Chymotrypsin	NH_2 | His(ser,glu,gly,thr,phe) thr(ser,asp,tyr) ser(lys,tyr) leu(asp,ser,arg,arg,ala,glu,asp,phe) NH_2 | val(glu,try) leu(met,asp,thr) ala(glu,asp,phe,val,glu,try) leu(met,asp,thr)
Trypsin (50 hr.)	His(ser,glu,gly,thr,phe) thr(ser,asp,tyr,ser,lys) tyr(leu,asp,ser,arg) arg ala(glu,asp,phe,val,glu,try,leu,met,asp,thr)
Subtilisin	His(ser,glu) gly(thr,phe) thr.ser asp(tyr,ser) lys.tyr leu(asp,ser,arg) NH_2 | arg(ala,glu) asp phe val(glu,try) leu.met NH_2 | asp,thr
Acid degradation products	His ser(glu,gly) glu.gly thr.phe thr ser(asp,tyr) asp.tyr ser.asp ser.lys tyr tyr.leu leu.asp tyr(leu,asp) asp.ser ser.arg
Carboxy peptidase action	NH_2 NH_2 NH_2 | | | ala(glu,asp,phe,val,glu,try,leu,met,asp,thr)
Summary	NH_2 NH_2 NH_2 NH_2 | | | | His.ser.glu.gly.thr.phe.thr.ser.asp.tyr.ser.lys.tyr.leu.asp.ser.arg.arg.ala.glu.asp.phe.val glu.try.leu.met asp.thr

chain was deduced from analysis of fragments obtained from the hydrolysis of glucagon by acid, carboxypeptidase, trypsin, chymotrypsin, and subtilisin. The resulting small peptides were isolated in pure form, largely by chromatography on columns of Dowex 50 resin. Each peptide fragment was analyzed for amino acids, for the amino terminal residue, and in some cases for amino acid sequence. All the data are consistent and, without exception, provide a firm basis for the amino acid sequence of glucagon shown in Table 3-1.

Comparison of the structures of insulin and glucagon reveals little, if any, similarity. A better understanding of the structural features that are necessary for the action of both hormones may provide a basis for future comparison. The relationship between the chemical structure of glucagon and its biologic activity is obscure. Since none of the known degradation products of glucagon retains hyperglycemic activity, the integrity of most of the molecule seems to be required for physiologic activity. Future work may reveal a correlation between the high proportion of functional groupings in glucagon and the various metabolic effects caused by this small protein.

SUMMARY

Glucagon is a small, well-characterized protein, in all probability a second hormone of the pancreas. It has been isolated in highly purified, crystalline form and has been characterized as a straight chain polypeptide with a molecular weight of 3485, containing 29 amino acid residues. Knowledge of the structure of glucagon demonstrates beyond doubt that glucagon and insulin are distinct and decidedly different entities.

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Chapter 4A

INSULIN AND GLUCAGON SECRETION

Arnold Lazarow

In the present discussion the morphology of the specialized cells concerned with the secretion of insulin and glucagon will be considered first. Subsequently, the secretion of these hormonal substances will be discussed under the following separate headings: (a) the synthesis of the protein hormone by the specialized islet cells; (b) the storage of the hormone within the cell; (c) the release of the hormone from the cell; (d) the multiplicity of ways by which the secretion of the hormone can be influenced.

THE MORPHOLOGY OF THE ISLET TISSUE

Although the existence of different cell types within the islets of Langerhans was noted by earlier investigators, the work of Lane carried out under Bensley's direction clearly established the presence of distinct cell types which contained specific secretion granules that could be selectively stained. In some species such as the guinea pig, two distinct cell types could be differentiated on the basis of cell size and nuclear morphology; in other species, however, the size and nuclear morphologic differences are less clear. Initially two cell types, i.e., the α-cells

and β-cells, were characterized by Lane on the basis of selective granule stains. A third cell type was described in the islet tissue of the guinea pig by Bensley and this "C" cell is a nongranular cell. A fourth cell type, the Δ-cell, was described in the human pancreas by Bloom (Plate 4A-1).

In the studies reported by Lane, the granules in the α-cell were fixed by 70 per cent alcohol and they could be stained by Bensley's neutral gentian method. The β-cell granules were not stained when this fixative was used and they were assumed to have been dissolved by the 70 per cent alcohol during fixation. By contrast Lane found that the granules in the β-cells were preserved in tissues fixed with chrome sublimate and stained by Bensley's neutral gentian. Thus, depending upon the fixative employed, it was possible selectively to stain either the α-cells or the β-cell granules using the same neutral-gentian stain. Approximately three quarters of the cells in the islets were β-cells, the remainder were assumed to be α-cells. In subsequent studies Bensley, using osmic acid-bichromate fixed tissue, was able simultaneously to stain both the α-cells and β-cells in a single preparation by aniline acid fuchsin and methyl green; following this procedure the granules of the α-cells were stained red whereas those of the β-cells were stained lilac.

More recently Gomori has introduced several staining methods that

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PLATE 4A-1. This human pancreas was surgically removed from a 36-year-old female, J. A. (UMH 92-26-92), during the course of a splenectomy and partial pancreatectomy. Samples of tissue were placed in various fixatives in the operating room.

¶1A. Tissue, fixed in Bouin, was stained with diluted Delafield's hematoxylin and eosin. Original magnification of × 220 was reduced to × 173.

¶2A. Same as 1A at an original magnification of × 1200 and reduced to × 945 Acinar and islets cells are shown.

¶1B Tissue, fixed in Bouin, was stained using a modification of the aldehyde fuchsin method with ponceau as a counterstain Magnification × 220 reduced to × 173.

¶2B. Same 1B at a magnification of × 1200 (× 945). The α-cell granules are red. The β-cell granules are purple.

¶1C. Tissue, fixed in Zenker-formol (Helly), was stained with a modification of the Heidenhain-Mallory-Azan method using azocarmine, aniline blue, and orange G Magnification × 220 (× 173)

¶2C. Same as 1C; magnification × 1200 (× 945). The α-cell granules are deep red The β-cell cytoplasm is orange. The Δ-cell cytoplasm is blue.

¶1D. Tissue, fixed in 10 per cent formol, was stained with a modification of the *Gros-Schultze silver method Magnification* × *220* (× *173*).

¶2D. Same as 1D, magnification × 1200 (× 945). The α-cell granules are black.

(I am indebted to Dr. Anna-Mary Carpenter and Mrs. Anne Marie Hult of the Department of Anatomy, University of Minnesota, for the preparation of the material from which these photomicrographs were taken)

effectively differentiate the cell types within the islet tissue. These procedures involve a preliminary oxidation step in which the tissue sections are treated with potassium permanganate; this markedly improves the staining characteristics. In Gomori's chrome-hematoxylin-phloxine method, the α-cell cytoplasm is stained red with phloxine and the β-cell granules are stained blue with hematoxylin. In Gomori's aldehyde fuchsin procedure, which has proved to be one of the most reliable and widely used methods, the β-cell granules are stained a brilliant red-purple with the aldehyde fuchsin, whereas the α-cell granules are unstained. It should be emphasized that the concentration of permanganate and acidity used in the preliminary oxidation step is critical and it must be controlled if background staining of the tissue is to be avoided. Elastic fibers are also selectively stained by aldehyde fuchsin. The granules of the alpha cells can be stained by certain of the silver impregnation methods. In the early studies, where the pancreas was either perfused with silver nitrate or the tissue blocks were impregnated with this reagent, the α-cells were reported to reduce the silver ion to the metallic state. This suggests that the α-cells contain a reducing substance. In later studies, which have been carried out with formalin-fixed pancreas, the tissue sections were placed in a staining solution containing ammoniacal silver plus an added reducing agent. Under these conditions the ammoniacal silver ion is reduced to metallic silver by the constituents present in the staining solution and the resulting reduced metallic silver micells are subsequently deposited on appropriate surfaces within the tissue sections. Ferner, using one of the many modifications of the silver method, has published photomicrographs that clearly show selective staining of the α-cell granules. However, since this silver staining method is capricious the results are often inconstant and frequently only occasional sections, out of many trials, show successful staining of the α-cells. Other investigators using different modifications of the silver methods have been unable to stain α-cells.

Although it had been suggested that the α-cells in the islet tissue are related to the intestinal enterochromaffin cells, this suggestion was made because both cell types have the common property of silver staining. This suggested identity based on staining properties should be accepted with reservation, since the silver staining reaction is certainly not a very specific one. Furthermore, it should be emphasized that although some of the silver staining procedures stain both the α-cells and the enterochromaffin cells, other of the silver methods do not stain the α-cells even though they are adequate for the identification of the enterochromaffin.

The third cell type, or C-cell, observed by Bensley in the islet of the

guinea pig, did not contain any specific granules and these nongranular cells were considered to be possible precursors of the α-cell. This suggestion was made because the number of granules in the α-cell has been found to vary considerably from cell to cell, and because the nucleus of the C-cell resembled that of the α-cell. However, this relationship between the C-cell and the α-cell has not been clearly established.

The Δ-cell, described by Bloom in the human pancreas, was observed in sections stained by the Mallory (Heidenhain) azan method. With this staining procedure the α-cells are stained red, the β-cells orange-yellow, and the Δ-cells blue. The latter cells are seen scattered individually within the islet and they are present in small numbers. The Δ-cells have not been found in all species studied. Although Bloom believes that the delta cells differ from the nongranular C-cells described by Bensley in the guinea pig pancreas, little is known about the functional significance of the Δ-cell.

THE β-CELLS AS THE SOURCE OF INSULIN

The following lines of evidence lend support to the thesis that the beta cells secrete insulin: (1) The islets of Langerhans appear to be the source of the antidiabetogenic factor which is hormonal in nature. (2) Alterations in the β-cells are associated with the development of diabetes. (3) There appears to be a parallelism between the islet tissue and β-cell granule content on the one hand and the insulin content of the pancreas on the other.

The early experiments showed that whereas pancreatectomy produced diabetes, the transplantation of small portions of pancreas from the bowel to the skin prevented glycosuria in the depancreatized dog. When the transplanted pancreatic graft remnants were removed the glycosuria was re-established. Because the elimination of the external secretion of the pancreas with the consequent impairment of digestion did not play a role in diabetes, it was postulated that the transplanted islet tissue prevented diabetes because it provided an internal secretion acting through the blood stream. The duct ligation experiments likewise supported the thesis that islet tissue alone mediated the antiglycosuric function of the pancreas. For although duct ligation eliminated the outflow of the pancreatic juice and resulted in a degeneration of the acinar tissue, the secreting elements of the islets of Langerhans remained and glycosuria did not develop. However, diabetes did appear when the degenerated pancreatic remnant containing the functional elements of the islets of Langerhans was removed.

The work of Holmans and Allan, carried out some nine years prior

to the discovery of insulin, indicated that the β-cells were the source of the antidiabetic principle. These investigators studied the cytologic changes in the islet tissue following partial pancreatectomy. They found that, when 80 per cent of the pancreas was removed, diabetes appeared progressively over a period of weeks. Corresponding cytologic studies of the islet tissue indicated that although the α-cells showed no changes, the β-cells remaining in the pancreatic remnant showed progressive degranulation and hydropic degeneration. These progressive degenerative changes in the β-cells, leading ultimately to their disappearance, closely paralleled the development of the symptoms of diabetes. The subsequent finding that alloxan injection produced selective destruction of the β-cells and diabetes clearly substantiates this relationship between β-cell alterations and diabetes.

Subsequent to the isolation of insulin it was shown that the insulin content of the varying regions of the pancreas paralleled the islet content. Thus the tail of the pancreas, which contains a larger number of islets than does the head of the pancreas, likewise contains larger amounts of insulin. Similarly it was shown that large amounts of insulin could be extracted from the duct-ligated pancreas in which the acinar tissue had degenerated and only the ducts and islet tissue remained. The chemical isolation of insulin from the anatomically separated islet tissue of fish further supports the thesis that insulin is associated with the islet tissue.

More recent comparative studies of the insulin content of the pancreas (as measured by bio-assay procedures) and the β-cell granule content (as estimated cytologically using aldehyde fuchsin stained material) indicate that the insulin content closely parallels the number of β-cell granules within the cell. Thus it is clear that the β-cells elaborate insulin and that the β-cell granule presumably represents a storage form of this hormone.

THE SYNTHESIS OF INSULIN BY THE β-CELL

The insulin molecule, which consists of two component polypeptide chains joined together by cross linkages, has a molecular weight of 6,000. The molecule is illustrated in Figures 4A-1 and 4A-2 by two cross-linked spiral structures. The two component polypeptide chains in insulin have been designated by Sanger as the A and B chains, and they contain 21 and 30 amino acids respectively. The exact amino acid sequence within each chain has also been clearly established. The work of Pauling and others indicates that each of the polypeptide chains in the insulin molecule is folded into a compact spiral structure or helix

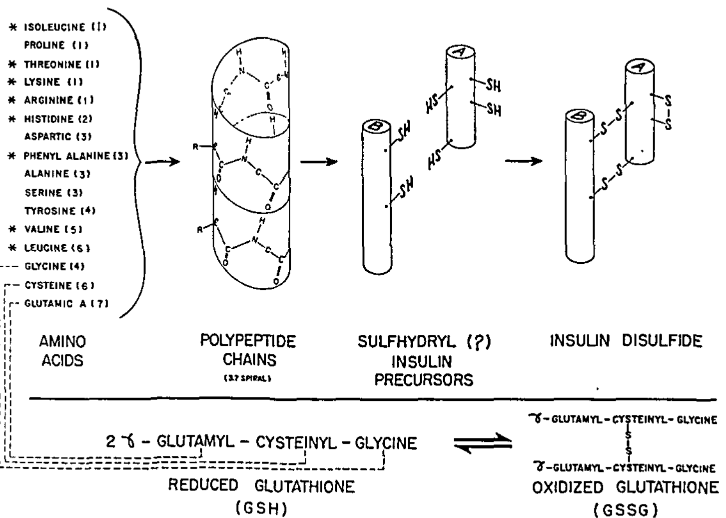


Fig. 4A-1. Synthesis of insulin.

(Fig. 4A-1). As a consequence the carbonyl group of a given amino acid is hydrogen bonded to the amide group of an amino acid four units along the chain (see Fig. 4A-1). Thus, hydrogen bonding provides the forces needed to hold the loops of the peptide spiral together. The two component helical polypeptide A and B chains of insulin are also joined together into a single molecule by two interchain disulfide bridges;

FIG. 4A-2. Metabolism of the beta cell: insulin synthesis, storage, and release.

these are located within the cystine residues spanning the two polypeptide chains. A third disulfide bridge extends between two of the loops of the A chain.

When insulin is synthesized by the β-cell, it may be assumed that the requisite number of amino acids are present simultaneously within the cell. Since some of these amino acids (indicated with an asterisk in Fig. 4A-1) are essential, i.e., they cannot be synthesized within the human body, they must be supplied in the diet. The nonessential amino

acids may be synthesized directly within the β-cell or they may be transferred to the β-cell following their synthesis at some other site such as the liver. It seems probable that the sulfur-containing amino acids are particularly important for insulin synthesis since they constitute 12 per cent of the amino acid residues in the insulin molecule. The sulfur in insulin must be supplied in the diet either as cystine (cysteine) or as methionine. The sulfur in methionine is converted into cysteine following transmethylation and the formation of a cystathionine intermediate; the cysteine thus formed is ultimately oxidized to cystine.

Practically nothing is known about the mechanism of insulin synthesis or about the enzymes within the β-cells that carry it out. Since the mitochondria are active metabolic sites within the cell they may contribute to insulin synthesis by supplying the energy needed for amino acid transformation and peptide bond synthesis. On the other hand the actual site of insulin synthesis may take place in the nonmitochondrial portions of the cytoplasm for it has been shown that in the liver protein synthesis can be demonstrated, using in vitro systems containing isolated cytoplasmic microsomal fractions.

It should be noted that the sulfur in insulin is in the disulfide form. Indeed the reduction of the disulfide groups of insulin to sulfhydryl results in a loss of insulin activity. It has been postulated on purely hypothetical grounds that the component A and B peptide chains of insulin might be synthesized separately, possibly as a sulfhydryl peptide chain, and that the two sulfhydryl peptide chains might be joined together enzymatically to form the insulin molecule by oxidizing the sulfhydryl groups to disulfide bonds (Fig. 4A-1). This would be analogous to the formation of oxidized glutathione in which two sulfhydryl-containing tripeptide molecules are joined together enzymatically in similar fashion by oxidizing their sulfhydryl groups with the formation of an interpeptide disulfide bridge. Unfortunately nothing is known about the enzymes that synthesize the specific peptide chains of insulin or about the factors that join or fold the peptide chains into the highly specific enzyme molecule. An interference in insulin synthesis could conceivably occur at any of the many stages in the process.

THE STORAGE OF INSULIN IN THE β-CELL

Although newly synthesized insulin may be excreted directly into the blood stream, insulin may likewise be stored within the β-cell for later release (Fig. 4A-2). The insulin that has been isolated from the pancreas by chemical means has a molecular weight of about 6,000 and is soluble at neutral pH. By contrast, granules containing insulin and pre-

sumed to be secretion granules can be centrifuged out of fish islet tissue homogenates. Thus the insulin or insulin precursor that is stored in the β-cell granule appears to be insoluble at the pH of the cell. Little is known about the way in which insulin is held within the secretion granule. Histochemical methods indicate that the islet tissue contains large amounts of zinc. Direct microchemical determinations show that the islet tissue of fish contains two to three times more zinc than does liver or kidney and that most of the zinc in islet tissue can be removed by centrifugation and recovered in the granule fraction. The addition of zinc to insulin solutions at neutral pH, in the absence of phosphate ion, markedly decreased the solubility of insulin. It has been shown that the insulin isolated from the pancreas by standard biochemical procedures likewise contains some zinc. Indeed, it has been suggested on the basis of physical chemical studies that the insulin monomers of molecular weight equal to 6,000 may be cross-linked into larger units by zinc; this is presumed to take place through metal binding of the histidine residues in the B chain of insulin. Although zinc may play a role in the aggregation of insulin, little is known about the precise nature of the ultrastructure of the secretion granules or the way in which insulin is held within a granule. It should be noted in passing, however, that α-cells may likewise contain large amounts of zinc and therefore the relationship between zinc, insulin, and the β-cell granule may be nonspecific.

RELEASE OF INSULIN FROM THE β-CELL

Although the β-cell can release its stored insulin into the pancreatic vein under appropriate stimulation, little is known about the mechanism by which the β-cell granule is disaggregated or the way in which the release of insulin is brought about. It has been shown that when the glucose level of the blood perfusing the isolated pancreas is increased to hyperglycemic levels, the pancreas responds by releasing insulin into the pancreatic vein. Likewise, the injection of glucose that produces a transitory elevation of the blood sugar is followed by a partial degranulation of the β-cells; the number of β-cell granules returns to normal as the blood sugar is restored. Thus, the release of the stored insulin from the β-cell appears to be associated with an active disaggregation of the β-cell granules and with the solubilization of the stored insulin. Although this solubilization may be a factor in providing for the rapid release of insulin into the blood stream, little is known about the mechanism by which it takes place or the way in which the solubilized protein molecules penetrate the surface of the cell. A better understand-

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Chapter 4B

ROLE OF ZINC IN INSULIN SECRETION

Helmut Maske

THE HISTOCHEMICAL DETECTION OF ZINC

In 1943 Okamoto demonstrated that the islets of Langerhans of many animals contained much zinc. Subsequently other intravital and post-mortem zinc stains with dithizone were developed, particularly with the use of frozen sections (Mager, McNary, Maske). These methods gave much better results, because extraction of zinc and insulin and destruction of original cell structures was reduced.

Zinc has been found regularly within the islets of humans, rabbits, dogs, rats, mice, cats, ducks, and various fishes (McNary, Maske, Okamoto, Weitzel). One exception among the animals studied so far is the guinea pig; in the islets of this species only traces of the metal, if any, can be demonstrated.

The distribution of zinc between the different cell types within the islets varies. In rabbits, dogs, and mice the metal may be found predominantly or even exclusively in the β-cells. In the islets of alloxan- or dithizone-diabetic rabbits with most of the β-cells destroyed, only traces of zinc can be found. In rats, β-cells contain small amounts of zinc, though α-cells contain much more. In ducks, where α-cell and β-cell-

islets are separate, the α-cells contain as much zinc as the β-cells (Runge).

WHAT IS THE FUNCTION OF ZINC IN THE ISLETS?

The presence of zinc in the islets is necessary for the very first phase of diabetogenesis with zinc combining substances. In this early stage, cytotoxic substances are concentrated within the cells and subsequently exert their action on the β-cells (Kadota, Okamoto). The fact that zinc-dithizonate formation within the α-cells of rats does not irritate those cells visibly points to a different physiologic function of the metal in the two types of cells (Maske).

The amount of histochemically demonstrated zinc within the β-cells varies under different conditions. After administration of glucose or adrenalin, considerably less zinc is found than is normally present. Prolonged glucose loading of the animal appears to eliminate most of the zinc (Maske, Wolff, and Stampfl).

These changes in dithizone-stained zinc-containing granules are similar to those that can be observed by employing conventional stains in β-cell granules under various physiologic conditions (Bell, Best, Campbell, Haist, and Ham). However, the granules demonstrated by dithizone appear to be greater, more distinct, and less numerous.

Moreover, the histochemically demonstrable zinc decreases after administration of glucose even before changes in the number of granules can be recognized by conventional histologic stains. After more intense glucose loading, zinc disappears from the islets, while granules can still be stained. Therefore, zinc represents a more sensitive indicator of functional alterations within the islets than do the granules as demonstrated with the usual histochemical methods (Maske).

THE INTRACELLULAR DISTRIBUTION OF INSULIN AND ZINC

Quantitative studies on the intracellular distribution of insulin and zinc have been made by homogenization and differential centrifugation of the giant islets of bony fishes (Maske). These results demonstrate that the highest concentrations of insulin and zinc are present in those fractions presumed to contain mitochondria and microsomes. The concentration of insulin in the fraction with the most of the hormone was found to be 4.7 per cent; though presumably the insulin content of the granules must be higher, considering the presence of mitochondria *and* granules within the same fraction. The concentration of zinc relative to that of insulin within the same fraction plus its washing fluid has been determined as 1.5 per cent; this is sufficient to precipitate pure insulin

or, and this appears more likely, to bind insulin to the proteins of granular cores.

Comparing the results on fishes with the histologic findings in mammals, the fraction containing the most insulin and relatively large amounts of zinc has to be related to granules that can be demonstrated histochemically with dithizone in respect to size and zinc content.

The most probable explanation for the difference in behavior between dithizone-stained granules in frozen sections and granules stained by the common histologic methods under different conditions is that the zinc that is attached to protein cores may disappear, while the granules persist.

ZINC AND INSULIN SECRETION

Insulin possesses a rather specific and strong ability to form chelate complexes with zinc and with some other metals (Cohn, Tanford). Above a pH of 6.0 insulin takes increasing amounts of zinc out of the surplus in solution. At the pH of normal blood, more than 2 per cent *zinc can be bound. Zinc binding has a strong influence on the solubility* of insulin (Cohn, Hallas-Møller). At pH above 6.0, insulin becomes insoluble if an excess of zinc is present, and if no stronger complex-forming substances are present. Under such conditions the metal is combined with the imidazole groups of the protein hormone. The insulin-bound zinc may still bind other basic groups, e.g., of amino acids, protamine, etc.

The rather specific and intensive ability of insulin to form insoluble complexes with zinc and the close intracellular relations between the hormone and the metal suggest that insulin is stored by the interaction of zinc within the granules. Before being secreted by the cell, insulin must be released from the granules.

The delivery of insulin is immediately dependent upon the glucose concentration in the pancreatic arteries. Investigations with triphenyltetrazolium chloride demonstrated a stimulation of islet cell metabolism after injection of glucose. Various metabolites such as citrate, oxalacetate, glutathione, cysteine, histidine, and organic phosphorus compounds form stronger complexes with zinc than they do with insulin. These substances can bring insoluble zinc-insulin complexes into solution by releasing the hormone. Within the cell they possibly act competitively in the same way, if greater amounts originate by an activated metabolism and by a greater supply of glucose than in resting cells. Therefore, an increased metabolism resulting from elevated blood glucose levels could stimulate the insulin secretion.

CONCLUSIONS

The delivery of insulin from the β-cells in response to blood sugar elevation may be pictured as follows: the stored hormone is firmly bound to proteins of granules. Zinc is essentially involved in this process.

When the blood sugar rises, the metabolism within the islet cells will be stimulated. The activation of the cell metabolism causes the release of insulin from the granules by changing the zinc bond of the hormone. A complex formation of various metabolites acting competitively with the zinc bound to insulin may be one of the mechanisms of this process.

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Chapter 5

ALPHA CELL CYTOTOXINS

Werner Creutzfeldt

Owing to the proximity of the exocrine and endocrine tissues in the mammalian pancreas and to the fact that the islets of Langerhans are composed of cells with different functions, surgical attempts to produce a glucagon deficiency by extirpation are highly problematic. In fact, they bring about conditions that are complex and difficult to survey. Moreover, selective partial pancreatectomy in the dog, in which only the uncinate process of the pancreas (which contains no α-cells) is left in the animal, did not lead to any abolition of function related to a glucagon deficiency (3).

There is, therefore, great interest in chemical substances that eliminate the α-cells. It should be possible to study the results of a glucagon deficiency in the intact animal with the aid of selective α-cell cytotoxins in analogy with the alloxan experiment that so splendidly allowed us to formulate our ideas about insulin production by the β-cells.

Morphologic changes occurring in the α-cells following the administration of various chemical substances have been described in recent years. The significance of these changes, as well as disturbances in carbohydrate metabolism that occur simultaneously, is discussed in some other chapters. The main problem is whether a causal association may possibly exist between the α-cell lesions and the disturbances in

carbohydrate metabolism, i.e., whether the latter can be related to a glucagon deficiency.

SODIUM DIETHYLDITHIOCARBAMATE (5, 12)

In 1951 Kadota and Midorikawa described a severe hypoglycemia preceded by an initial hyperglycemia that occurred after the intravenous administration of sodium diethyldithiocarbamate (NaDDC) in several rabbits. The histologic examination of the pancreatic islets revealed pyknosis of the nuclei, degeneration of the cytoplasm, and atrophy of the cells in these cases. The authors explained the hypoglycemia by the destruction of the α-cells. Subsequent investigators, however, were not able to confirm the α-cell lesions following the administration of the NaDDC, although in a few cases severe hypoglycemia was similarly observed (12). NaDDC is a highly toxic substance and might affect various enzyme reactions by virtue of its reaction with numerous metals.

In view of the findings described, NaDDC cannot be regarded as an α-cell cytotoxin.

COBALTOUS CHLORIDE (5, 7, 11, 23)

In 1951 Van Campenhout and Cornells (21) observed an impressive degranulation and vacuolization of the α-cells following the subcutaneous administration of 10 to 15 mg./kg. $CoCl_2$ for several days in the guinea pig. The finding was confirmed in numerous laboratories for this species. Figure 5-1 shows a pancreatic islet from a guinea pig with grossly swollen α-cells that appear empty but have intact nuclei. Granules are still present in several vacuolated α-cells. The histochemical reaction for fat and glycogen is negative in the vacuoles.

Destruction of the damaged α-cells only rarely occurs, and after the administration of $CoCl_2$ is stopped the cells recover within a few days. It is very questionable whether the alterations in the α-cells arise through direct cell damage. The gradual development of the changes in the course of several days argues much more in favor of a secondary degeneration due to hyperactivity (7). The observation that chronic administration of not too high doses of $CoCl_2$ leads to an increase of α-cells (15) might be explained in the same way. The cause of a raised glucagon requirement during $CoCl_2$ poisoning is unknown. Various enzyme systems are inhibited by cobalt salts in vitro (Levy, von Euler). The doses of $CoCl_2$ necessary to produce α-cell damage are already highly toxic and frequently lead to the death of the animals. Degenerative changes can be demonstrated in various organs.

It is seldom that *all* α-cells are damaged after the administration of

$CoCl_2$ in the guinea pig. The average glucagon content in the pancreas is distinctly reduced, according to various investigations, and in cases of very severe α-cell damage no further hyperglycemic activity can be detected (2). On the other hand, a fall in the blood sugar to hypoglycemic values was never observed, even in the most severe cases of α-cell damage in the guinea pig.

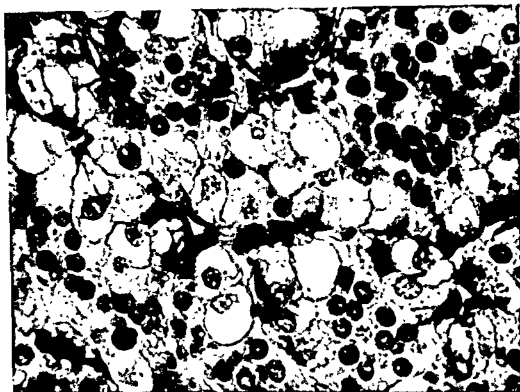


FIG. 5-1. Section through a pancreatic islet from a guinea pig which had been given 10 mg./kg. $CoCl_2$ subcutaneously for 3 days. Severe α-cell hydrops. (Bouin, staining with Azan after Gomori, × 300, Leica.) See Chapter 25 (Fig. 25-17) for electron miscroscopic appearances after cobalt.

In teleostea the colloid droplets in the α-cells characteristic for these species disappear under $CoCl_2$, while the blood sugar raising effect of islet extract simultaneously declines (Mosca). In other types of animal the effect of $CoCl_2$ is less impressive and very inconstant (11). Controversies have, therefore, arisen in the literature without the problem of α-cell function being elucidated.

At any rate, the investigations of Volk, Lazarus, and Goldner cannot be passed over. They described a complete disappearance of the α-cells in the rabbit and dog following administration of $CoCl_2$, without a fall

in the glucagon content of the pancreas or hypoglycemia simultaneously occurring. This question requires further clarification.

It remains to be mentioned that the initial hyperglycemia occurring after the administration of $CoCl_2$ cannot be related to the glucagon release of damaged α-cells. The rise in blood sugar level can in fact be produced with very small doses of $CoCl_2$, which certainly do not affect the α-cells. It also occurs in the pancreatectomized animal and is absent after administration of dihydroergotamine as well as after adrenalectomy.

Nickelous chloride acts like $CoCl_2$ on the blood sugar and the α-cells. The α-cell changes are not as impressive, however, as those that occur after the administration of $CoCl_2$.

In summary, it appears that $CoCl_2$ causes morphologically impressive changes in the α-cells of the guinea pig in the course of a few days (secondary degeneration?). The glucagon content of the pancreas decreases parallel to the severity of the α-cell damage; the blood sugar, however, does not fall to hypoglycemic values. The effect of $CoCl_2$ on the α-cells in other species is inconstant. The substance has a complex action on various enzyme reactions.

GUANIDINE DERIVATIVES

Synthalin A (5, 8, 9, 16, 17)

In 1952, Davis announced that, following subcutaneous injection of 6 to 9 mg./kg. synthalin A (decamethylenediguanidine) in the rabbit, after 12 to 15 hours a degranulation and vacuolization of a variable part of the α-cells occur. This observation was confirmed by various subsequent investigators on both normal and alloxan diabetic rabbits. Figure 5-2 shows vacuolated, degranulated, and intact α-cells in a pancreatic islet from a rabbit 36 hours after subcutaneous injection of 6 mg./kg. synthalin A. The picture demonstrates very well the actual conditions, because the α-cell alterations are extraordinarily inconstant in the rabbit. Fat and glycogen could not be detected in the vacuolated cells as in $CoCl_2$ poisoning. Pancreatic extracts from synthalin-treated rabbits possessed no further hyperglycemic and glycogenolytic action (Fodden, Read).

The α-cell changes seen in the guinea pig when 3 mg./kg. of synthalin A is injected subcutaneously are more impressive and constant than those that occur in the rabbit. Figure 5-3 shows the complete α-cell hydrops that occurs in a guinea pig treated in this way. Distinct α-cell changes also occur in rats following the administration of synthalin. However, they consist of a high-grade degranulation only, without typical cell hydrops.

Von Holt and co-workers described a complete degeneration of the α-cells and called synthalin A a primary α-cell cytotoxin. Other authors concluded, on the other hand, from the slow development of the α-cell changes and the absence of α-cell necrosis in their experiments, that only an exhaustion degeneration was involved on the part of the α-cells (8, 9). As is well known, purely from the morphologic point of view there is no difference between the results of excessive functional demands and cytotoxic damage in many secretory cells.

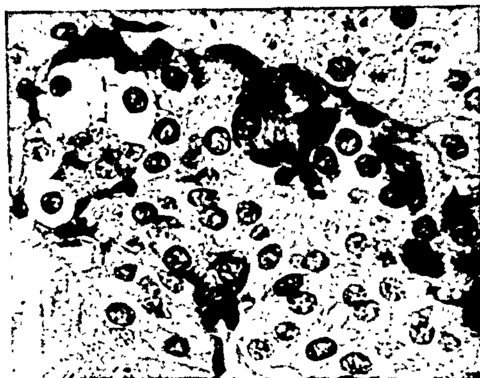


FIG. 5-2. Pancreatic islet of a rabbit 36 hours after subcutaneous injection of 6 mg./kg. synthalin A. Degranulated and vacuolated α-cells in addition to normal ones (dark) (technique as in Fig. 5-1).

The answer to this question in dispute is of great interest, because synthalin A, in contrast to $CoCl_2$, produces first an initial hyperglycemia and then leads to severe and usually fatal hypoglycemia. In accordance with their interpretation of the α-cell changes as toxic cellular death, von Holt and co-workers explain the fall in blood sugar that occurs after synthalin as the result of a glucagon deficiency and evaluate the synthalin experiment as a proof of the important role that the α-cells play in blood sugar homeostasis. The other authors regard the α-cell changes as a side effect of synthalin A, and assert their opinion that the degree of α-cell damage seldom runs parallel to the severity of the hypoglycemia. While the fall in blood sugar level following the ad-

ministration of synthalin A occurs regularly, the α-cell changes are inconstant. Moreover, it has not been found possible to prevent the synthalin hypoglycemia by means of continuous injections of glucagon (8).

Both old and new physiologic and biochemical findings, according to which the substance has an extra-pancreatic site of action, argue against the conception that synthalin A acts to cause a lowering of the

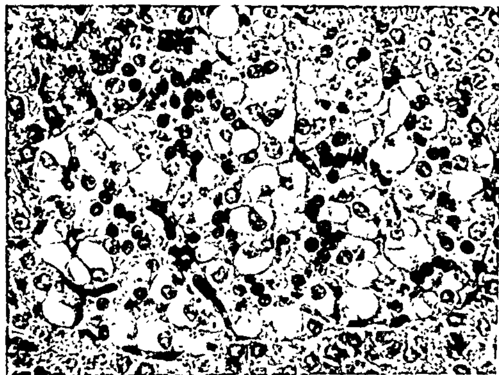


Fig. 5-3. Pancreatic islet of a guinea pig after injection of 3 mg./kg. synthalin A for 3 days. High grade α-cell hydrops. (× 200, otherwise technique as in Fig. 5-1.)

blood sugar by effecting a disturbance of the α-cells. Synthalin A lowers the blood sugar even in pancreatectomized and eviscerated animals. It inhibits oxygen consumption and raises anaerobic glycolysis (4, 20). In vitro the step is inhibited that links oxidation and phosphorylation, in fact at the stage of cytochrome C (14). Moreover, synthalin A, when given in doses that will reliably cause a fall in blood sugar, possesses a considerable organ toxicity for the liver and the kidneys. The adrenal cortex is powerfully activated at the same time and in the adrenal medulla exhaustion degenerations occur.

The observations mentioned favor the assumption that in the course

of the synthalin poisoning an abnormally increased glucagon requirement occurs, whereupon a variable part of the α-cells undergo secondary degeneration.

Synthalin B (6, 9)

Synthalin B (Dodecamethylenediguanidine) does not cause α-cell changes in rabbits in spite of causing hepatic and renal damage (9).

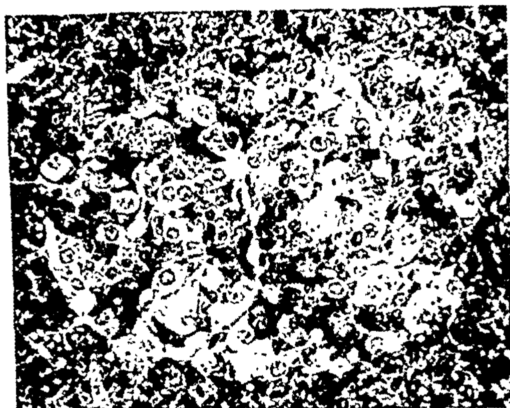


Fig. 5-4. Pancreatic islet of a guinea pig following injections of 3 mg./kg. synthalin B for 3 days. Marked degranulation and partial vacuolization of the α-cells. The β-cells (dark) are normally granulated. (Boulin, staining with aldehydfuchsinphloxin, × 200, Leica.)

In guinea pigs, however, degranulation and vacuolization of the α-cells can be produced with synthalin B (6). Figure 5-4 shows marked alterations in the α-cells of the pancreatic islet in a guinea pig following injection of 3 mg /kg. synthalin B for 3 days. On the whole, the effect of synthalin B poisoning on the α-cells seems to be somewhat weaker than is the case with synthalin A. As regards the fall in blood sugar, the rise in lactic acid in the blood, and the hepatic and renal necroses, no fundamental differences are obvious between synthalin A and B.

The observation that the rabbit, in contrast to the guinea pig, shows

no α-cell changes after synthalin B only confirms the experience gained from $CoCl_2$ that the α-cell system of the guinea pig reacts in a more sensitive manner than that of other species.

Phenylethyldiguanide (6)

After the introduction of the diguanides into the therapy of diabetes (see Chap. 35) the question arose as to whether morphologic changes occurred in the α-cells after the administration of these blood sugar

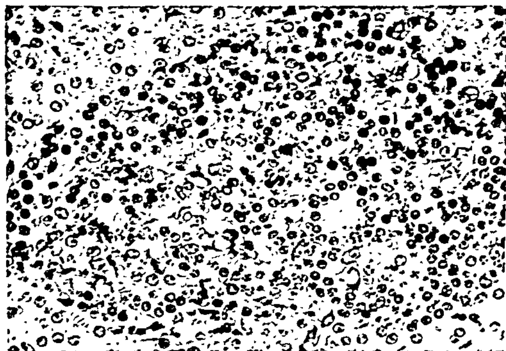


FIG. 5-5. Pancreatic islet of a guinea pig following injections of 15 to 25 mg./kg. DBI for 3 days. Complete degranulation of the α-cells, which normally stand out dark with this staining technique (compare Fig. 5-6). (× 200, otherwise technique as in Fig. 5-1)

lowering guanidine derivatives. Physiologic and biochemical investigations hitherto showed that the mechanism of action of the diguanides is fundamentally identical with that of synthalin (24, 25). The toxicity is considerably lower, however (Ungar).

Frequently, degranulation and sometimes vacuolization of the α-cells in the guinea pig can be observed following the administration of phenylethyldiguanide (DBI) in doses that certainly bring about a reduction in blood sugar (15 to 25 mg./kg. subcutaneously over 3 days) (6). Figure 5-5 shows the comple[illegible] ulation of the α-cells following the administration of DBI i[illegible] ea pig and Figure 5-6, for

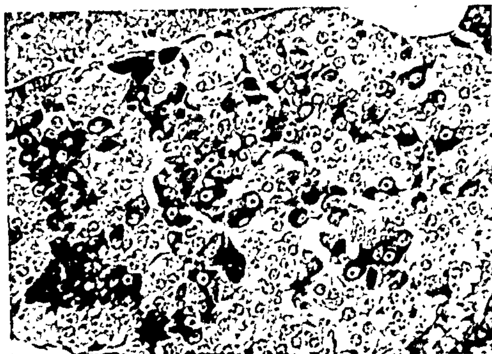


Fig. 5-6. Pancreatic islet of a normal guinea pig. The α-cells, which appear red in the original preparation, appear dark in the picture. (× 200, otherwise technique as in Fig. 5-1.)

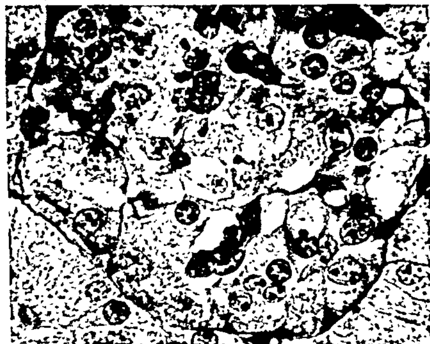


Fig. 5-7. Pancreatic islet of a guinea pig following injection of 10 to 20 mg./kg. DBI for 3 days. Distinct vacuolization of the α-cells. (× 500, otherwise technique as in Fig. 5-1.)

comparison, shows a normal islet with the same staining method. In Figure 5-7 a vacuolization of α-cells can be recognized following the administration of DBI in the guinea pig. The changes do not differ qualitatively from the synthalin effect, but they are distinctly less constant although the fall in blood sugar occurred regularly in the DBI dosage employed.

In summary, it appears from a comparison of the various guanidine derivatives that the α-cell changes cannot be the cause of the guanidine hypoglycemia but simply represent a side effect whose severity differs within the various substances. Probably in the course of the poisoning a raised glucagon need occurs with subsequent secondary α-cell degeneration. The fall in the blood sugar is not responsible for this since it is the same with the various guanidine derivatives. Possibly, however, the varying degree of hepatic metabolic disturbance leads to an abnormally increased glucagon secretion.

LIVER CELL POISONS (5, 8, 22)

Verne had already described α-cell alterations in rats following poisoning with *Amanita phalloides* in 1949. In the guinea pig, almost

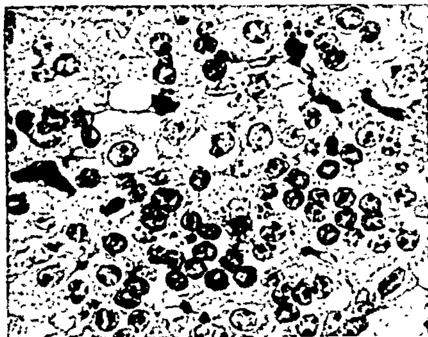


Fig. 5-8. Pancreatic islet of a guinea pig following injection of 10 mg./kg. phosphorus for 3 days; α-cell hydrops and complete degranulation of the α-cells. (× 500, otherwise technique as in Fig. 5-1.)

regular degranulations, frequent vacuolization, and increased mitotic activity of the α-cells could be demonstrated following the administration of various liver poisons such as phosphorus, carbon tetrachloride, chloroform, and ethionine (8). Figure 5-8 shows the vacuolated α-cells of a guinea pig following three days of injections of 10 mg./kg. phosphorus. Destruction of the α-cells was never observed. The alterations are again best interpreted as a sign of an increased α-cell activity as a result of a raised glucagon requirement in the course of liver damage. Not every liver damage leads to α-cell changes, however.

BLOOD SUGAR LOWERING SULFONAMIDES (19)

Von Holt and co-workers described severe α-cell lesions following the administration of p-aminobenzolsulfonamideisopropylthiodiazole (IPTD) and used these to explain the blood sugar lowering effect. Other authors found only transitory degenerative changes (or none at all) in the α-cells of rabbits and rats following the administration of IPTD (1, 8, 13, 19). The glucagon content of the pancreas was normal after the administration of IPTD (Vuylsteke and De Duve). The general opinion is that the blood sugar lowering effect of IPTD probably depends on its beta cytotropic property (Loubatieres).

As regards the action of the sulfonylureas (carbutamide and tolbutamide, see Chap. 35) at first presumed to be based on α-cell damage, conclusive proof is lacking in spite of numerous investigations. The occasionally observed transitory α-cell changes (granular clumping and partial degranulation) in experimental animals (10) cannot be regarded as pathologic. Diabetics treated with sulfonylureas did not show any degree of α-cell changes at post-mortem.

CONCLUSIONS

Of the various substances that become known as α-cell cytotoxins, only cobaltous chloride and certain guanidine derivatives produce significant changes in the α-cells. The α-cell changes in the guinea pig are most easily produced. There is no evidence so far for the assumption that the α-cell changes depend upon a primary cytotoxic property of the substances named. It is more probable that, following the administration of the so-called α-cell cytotoxins, an increased glucagon secretion occurs with consecutive exhaustion degeneration. Disturbances of liver metabolism may play a causal role in this connection, because certain liver cell poisons likewise lead to secondary α-cell changes. Owing to the complex action of the presently known α-cell cytotoxins, it is not possible to produce a glucagon deficiency syndrome with these sub-

stances. A further search for *specific* α-cell cytotoxins is, therefore, desirable.

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Chapter 6

INSULIN AND GLUCAGON DISTRIBUTION

Robert H. Williams

Methods for measuring the distribution of insulin and glucagon throughout the body have included bio-assays, electrophoresis, chromatography, and localization of radioactivity of labeled hormone. Each of these methods is beset with significant difficulties. As discussed in other chapters, there are major problems in the quantitative extraction of these hormones from body tissues and fluids. Bio-assay in the presence of certain other body constituents presents a summation of stimulative and inhibitory effects. Moreover, some of these substances interfere with electrophoretic and chromatographic assays.

I^{131} is the isotope that has been used most frequently for labeling insulin and glucagon. With heavy iodination, or without appropriate care in other ways, the chemical characteristics and biologic activity of these hormones may be altered significantly, thereby giving misleading information relative to the distribution of the respective hormone. Moreover, there are slight species differences in the chemical composition of the hormones. There also is a question as to whether exogenous insulin and glucagon have exactly the same chemical composition as endogenous products in the same species. The distribution of these hormones varies with the amounts and sites of injection, with their rate of deg-

radation, with the amount of certain allied proteins present, and with many other factors. Thus, many studies of the distribution of these hormones must be accepted with considerable reservation, but it is believed that a great deal of useful information has been obtained. Many of the earlier outstanding studies of Stadie and others have been reviewed recently (Williams).

PLASMA AND EXCRETA

Following the intravenous injection of insulin, it rapidly disappears from plasma (Fig. 6-1). Its distribution has been stated to conform chiefly to that of extracellular fluid (Wick, Prout). Indeed, it has been claimed that a summation of its extracellular distribution and degradation products can account for most of the injected hormone. Its disappearance is much faster in normals than in insulin-treated subjects; after several weeks of insulin therapy antibodies accumulate in the plasma, binding the insulin. Berson has demonstrated "antibody" in the α, β, and γ globulin fractions, and in albumin; when complexed with insulin the electrophoretic mobility is chiefly in the γ-β globulin interzone. There is a rapid and a slow complexing fraction; the rapid-binding material releases insulin faster than the slow fraction. While insulin is complexed with antibody it tends to be protected from degradation, preserving its biologic activity. In this manner more than 500 units of insulin may be transported in each liter of plasma. In some instances the binding is so persistent that several thousand units per day may be required for proper control of diabetes. Following this massive storage of insulin it is sometimes freed rapidly, causing severe hypoglycemia. Under certain conditions insulin may bind to other plasma protein fractions. It also binds readily to paper, glass, and tissues. Goodner found significantly different concentrations of insulin in Cohn Fractions of plasma. Moreover, he found that insulin degradation by liver homogenate was inhibited by certain dialyzed Cohn Fractions of plasma (III > IV-1 > II-III = IV-4).

On the basis of electrophoretic, chromatographic, and/or assay studies Berson, Scott, and Prout have reported that some of their radioiodinated insulin preparations have contained altered as well as unaltered insulin and the stronger the radioactivity and the more prolonged the exposure the more the alteration. Intense radiation has been shown to split disulfide bonds of insulin, causing a loss of hypoglycemic action. It had been previously shown that when reducing agents split as many as one third of the disulfide bonds, biologic activity may be abolished. With paper electrophoresis the unaltered fraction of radioiodinated in-

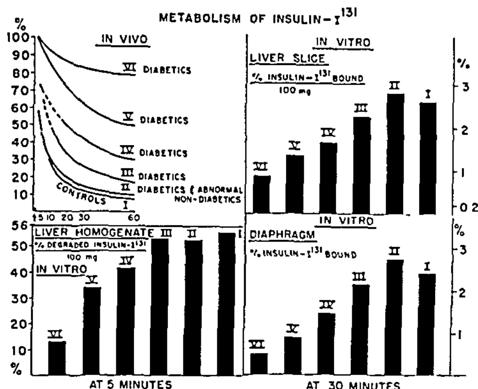


FIG. 6-1. The left upper block shows the percentage of insulin-I131 injected intravenously that remained at different intervals within the next 60 minutes in 43 nondiabetics and 63 diabetics. For convenience the subjects were grouped. Group I was composed of 7 normal subjects. Group II consisted of 6 patients who never received insulin, 12 who had received insulin for more than 6 weeks and 32 patients with various nondiabetic disorders. Groups III–VI consisted of diabetics who had been treated with insulin, as well as of 2 nondiabetics who never received insulin

The left lower block shows that the rate of degradation of insulin-I131 when incubated with patient plasma and rat liver homogenate was inversely proportional to the amount of insulin-I131 retained in the plasma in in vivo experiments. The two blocks on the right show inverse relationships of the amount of insulin retained in the plasma in vivo and the amount of insulin-I131 transferred from the plasma to rat liver slices or diaphragm Thus, as discussed in the text, when insulin was bound to plasma globulin there was a decrease in its rate of transfer to tissue and a decrease in degradation by insulinase (After Welsh, G. W , III, Henley, E. D., Williams, R. H., and Cox, R W. *Am. J. Med. 21*:324, 1956)

sulin remains at the point of application, but the altered hormone is said to migrate with the plasma proteins, moving at about the same rate as albumin; it also is precipitable with trichloroacetic acid (TCA).* Berson found with starch electrophoresis that insulin was not adsorbed significantly to the starch; in normal serum it migrated with a mobility almost as great as that of albumin. With ultracentrifugation it sedimented less rapidly than albumin in normal serum but with the globulins in the sera of insulin-treated subjects. The altered insulin disappeared much less rapidly from the plasma than did the unaltered and was less subject to further degradation. Moreover, it was reported to be biologically inactive (Scott); the total biologic activity correlated with the amount of unaltered insulin. The aforementioned observations must be considered very seriously but it has not been proved that the portion of freshly prepared insulin-I^{131} migrating upon paper electrophoresis is inactive. There are many factors, particularly protein binding, that influence electrophoretic mobility and insulin bio-assay, which apparently occurs in this situation. Great caution must be used in avoiding excessive iodination and irradiation of the hormone. In some studies it is necessary to utilize a freshly prepared and freshly dialyzed preparation. It is of interest that Lee found by gentle treatment of insulin with alkali that the hypoglycemic action was destroyed and that the degraded material disappeared from the plasma much *faster* than the undegraded.

As discussed in Chapter 31, normal plasma contains only a minute amount of insulin and juvenile diabetics have only a small proportion of the normal quantity. Adult-onset diabetics, though usually having a somewhat subnormal concentration, occasionally have a normal or hypernormal level. There is normally a severalfold increase with a glucose load; fatty acids and amino acids also increase the plasma concentration of insulin.

Following intravenous injection, glucagon-I^{131} disappears from the plasma much faster than insulin-I^{131}; it also is degraded much more rapidly. Although assays for nonlabeled glucagon in purified form have been satisfactory, assays of body tissues and fluid have not been adequate. No significant difference in plasma concentration of TCA-precipitable radioactivity has been observed whether insulin-I^{131} and glucagon-I^{131} is injected into the portal vein or tail vein of rats. Only a

* It has been demonstrated that TCA solubility of radioactivity can be used as an indication of the amount of degradation of insulin-I^{131} and glucagon-I^{131}. When these hormones undergo slight degradative changes they may be TCA-precipitable but in the studies reported herein it is believed that this probably has constituted only a very small proportion of the total amount of degraded hormone.

very minute amount of insulin or glucagon is excreted in the urine and bile.

TISSUE CONCENTRATION

Most of the conclusions relative to the localization of insulin and glucagon in tissues are based upon measurements of protein-bound radioactivity following injections of these hormones labeled with I^{131}. It is not known how much of the measured radioactivity consists of undegraded hormone, but accumulated evidence suggests that intracellular penetration of insulin and glucagon occurs. Most of the enzyme degrading system for these hormones is in the fraction remaining after ultracentrifugation of liver homogenate. Both hormones rapidly enter most of the tissues throughout the body and are rapidly degraded; most of the tissues of the body have been shown (Williams *et al.*) to exhibit this degradative action, with liver, pancreas, kidney, and testis being among the most active. Since the amount of undegraded hormone remaining in a given tissue is dependent so much upon the rate of hormone degradation, a brief review of this phenomenon is presented now, but fuller discussions are found in Chapters 21 and 22.

In the case of liver, insulin is apparently degraded by enzymatic and nonenzymatic reduction as well as by proteolysis, whereas with glucagon, containing no disulfide linkages, degradation is by only the latter process. It has been suggested by Tomizawa and Narahara that other disulfide-containing hormones, e.g., somatotropin, prolactin, oxytocin, and vasopressin, may, like insulin, be degraded by both reductive and proteolytic reactions whereas the degradation of corticotropin, like that of glucagon, would involve only proteolysis (Fig. 6-2). Whereas glutathione inactivates insulin directly by reduction, this action apparently is catalyzed by a liver enzyme (Narahara); moreover, glutathione reductase helps maintain the supply of reduced glutathione. It is not known to what extent the degradating mechanisms in other tissues resemble those of liver, but some similarities have been demonstrated in skeletal muscle and pituitary (Narahara).

Several proteins have been demonstrated in vitro to spare the degradation of insulin and glucagon; among these are somatotropin, corticotropin, and casein. Also, insulin and glucagon spare the degradation of one another. When huge doses of insulin are injected intravenously with insulin-I^{131} there is a decreased uptake of the latter by the tissues. A minute amount of glucagon, too small to influence glycogenolysis, in the presence of insulin, ACTH, or somatotropin exerts a significant glycogenolytic effect on liver slices (Tyberghein). Similarly, insulin stimulation of the glucose uptake by diaphragm is enhanced by the

presence of these hormones. The increased actions result from sparing of the degradation of insulin or glucagon.

The following insulin-I[131] distribution studies were conducted in rats by Elgee *et al.* with a preparation similar to one which maintained full biologic activity as measured by hypoglycemia responses in rabbits and mice and by glucose uptake by rat diaphragm. It also had the same mobility as unlabeled insulin in paper electrophoresis. From 0.2 to 0.8 units (10 μc or less) was injected intravenously into each rat. As shown in Figure 6-3A, there was a marked variation in the different tissues in trichloroacetic acid precipitable and nonprecipitable (degraded insulin)

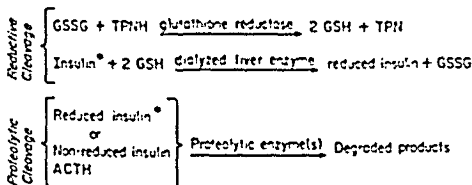


FIG. 6-2. (After Williams, R. H., Hay, J. S., and Tjaden, M. B.: *Ann. New York Acad. Sc.* 1959.)

radioactivity. Brain and red blood cells had essentially no radioactivity—an interesting observation in view of the fact that insulin exerts no action in these tissues. Kidney and liver had the highest concentrations and they, along with skeletal muscle and blood, had the largest total quantity of radioactivity, 61 per cent being in these four tissues.

Comparable studies with glucagon-I[131] (Fig. 6-3B) and ribonuclease-I[131], revealed similar patterns of distribution. Indeed, investigations by others have revealed similarities with ACTH-I[131] and prolactin-I[131].

The very high concentration of these compounds in the kidneys prompted special studies by Narahara. He found, within 2 minutes following the intravenous administration of glucagon-I[131] or insulin-I[131], significant accumulation of labeled protein in the lumina of the proximal convoluted tubules with subsequent collection of radioactivity in the

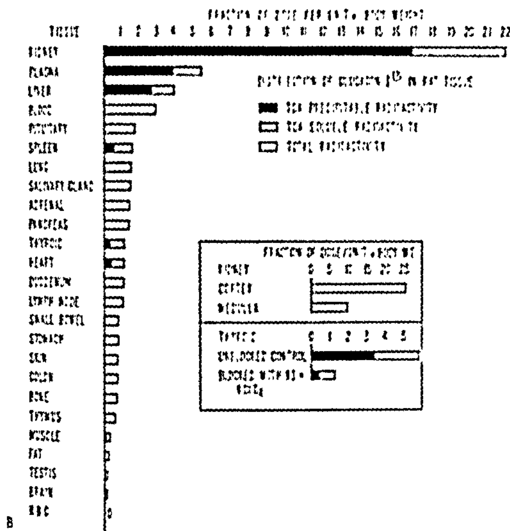


FIG. 6-3B. Distribution of radioactivity 5 minutes after tail vein injection of each of 8 rats with 23 µg. of glucagon-I^{131}. All concentration values greater than unity represent localization in the specific tissue greater than the theoretical concentration in the total body. The pattern of glucagon-I^{131} distribution is similar to that of insulin-I^{131}. The percentage of the total injected radioactivity was: kidney 15 per cent, muscle 12 per cent, liver 12 per cent, and plasma 8 per cent; the remainder was scattered among the other tissues and fluids. (After Cox, R. W., Henley, E. D., Narahara, H. T., VanArsdel, P. P., Jr., and Williams, R. H.: *Endocrinology 60* 277, 1957.)

proximal cells (Fig. 6-4). However, there was a virtual absence of radioactively labeled protein in the more distal tubules and in the urine. Supposedly, these small protein hormones passed rapidly through "pores" in the glomerular membranes, but were rapidly reabsorbed in the proximal convoluted tubules. However, little is known about the metabolism of these hormones in the kidney.

Time curves for the disappearance of TCA-precipitable and non-precipitable activity are shown in Figure 6-5. The concentration of TCA-precipitable radioactivity reached a peak in the liver, kidney and muscle in from 5 to 15 minutes and then fell rapidly, paralleling that in

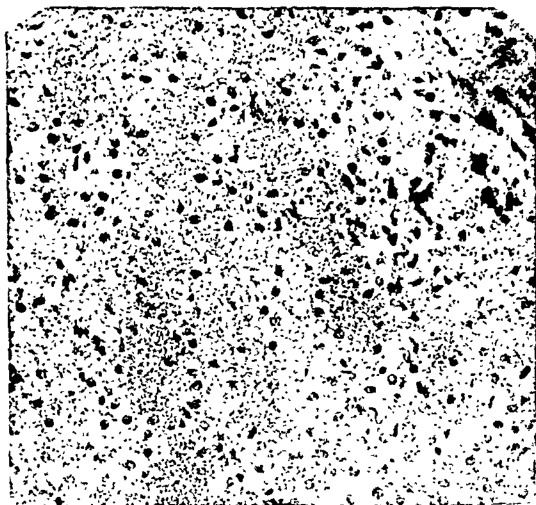


FIG. 6-4A. Photomicrograph of radioautograph of rat kidney. Cortex of kidney excised 60 sec. after injection of insulin-I^{131}. 5 μ section, 24-hr. exposure. Note that the reduced silver overlies the lumen and cells of a proximal convoluted tubule. (× 290.) (Figs 6-4*A*–*D* from Narahara, H T, Everett, N B., Simmons, B S., and Williams, R H. *Am J Physiol* *192* 227, 1958.)

blood; after 1 hour the fall was slight. TCA-soluble radioactivity, indicative of degraded insulin, appeared within the first few minutes after insulin-I^{131} injection and increased in concentration in the tissues and blood in the first 15 minutes. Chromatographic studies showed that

very little radioactivity was in the form of iodide, apparently most of it was attached to peptides.

Lee and Williams found that insulin-I^{131} apparently entered cells rapidly and was distributed in the intracellular components in a characteristic manner. After equilibrium was reached in the liver and kidney,



FIG. 6-4B. Photomicrograph of radioautograph of rat kidney. Cortex and outer medulla of kidney excised 6 minutes following administration of insulin-I^{131}. 6 μ section, 30-hr. exposure. Note the ring-shaped area of reduction overlying some of the proximal convoluted tubules. Observe that the more distal segments of the nephron are essentially free of radioactivity. ($\times$ 87.)

the concentration of radioactivity was found in the following decreasing order in cellular components separated by ultracentrifugation: residual fraction, mitochondrial fraction, nuclear fraction, and microsomal fraction; the amount in the microsomes of the kidney was relatively very small. When insulin-I^{131} was added to liver homogenate a much smaller quantity was fixed to the intracellular components than when it was

administered in vivo. This could be attributed to the importance of cellular integrity for the concentration of the hormone, but it is also related to the increased rate of insulin degradation produced by homogenization.

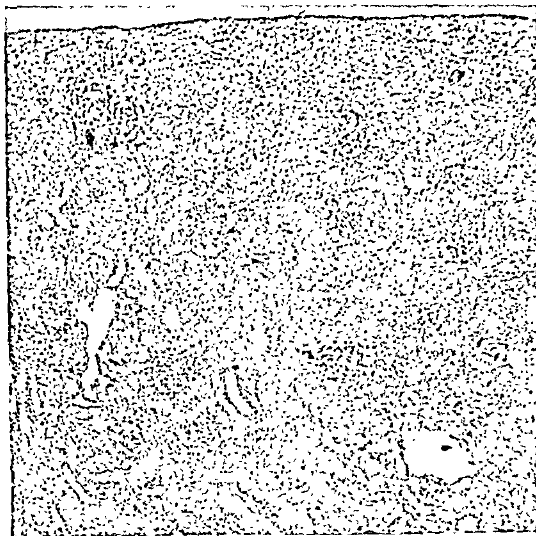


Fig. 6-4C. Photomicrograph of radioautograph of rat kidney. Cortex and outer medulla of kidney removed 10 sec. after the injection of glucagon-I^{131}. 6 μ section, 7-day exposure. Note that even at this early interval there is a localization of radioactive material over certain of the proximal convoluted tubules (× 87)

Perfusion of the liver and kidney failed to decrease significantly the concentration of radioactivity after insulin-I^{131} injection, but not after Na I^{131}, albumin-I^{131}, or thyroxin-I^{131}. Stadie and colleagues found that insulin-I^{131} became bound to diaphragm, breast, adipose tissue, leuko-

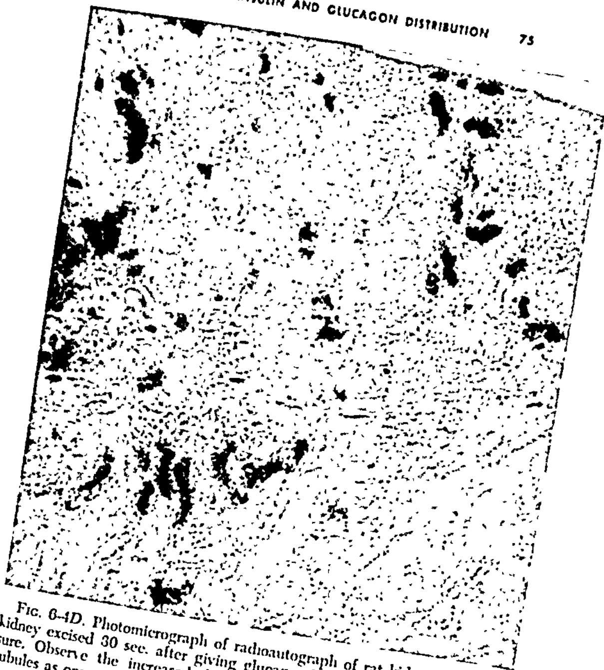


FIG. 6-4D. Photomicrograph of radioautograph of rat kidney. Cortex of kidney excised 30 sec. after giving glucagon-I^{131}. 6 μ section, 7-day exposure. Observe the increased density overlying the proximal convoluted tubules as opposed to that seen in the 10-second interval. The distribution is comparable to that seen with insulin-I^{131}. (× 87.)

cytes, erythrocytes, thymus, spermatozoa, and bone marrow. They reported that insulin became bound to diaphragm within a few seconds and remained bound despite repeated and prolonged washing. Diaphragm bound only 1/50 as much as leukocytes and erythrocytes only 1/1000 as much as leukocytes, the amount bound was dependent upon its concentration. There was a linear correlation of the amount of insulin-I^{131} bound to diaphragm with the rate of glycogenesis. It was

suggested that insulin may combine with enzyme and thereby influence substrate-enzyme reactions in a manner comparable to that postulated by the Michaelis-Menten concept of substrates combining with enzymes.

Investigations in this laboratory have shown that most of the tissues of the body tend to bind insulin-I^{131}, but with repeated washing we have observed that there was a progressive decrease in tissue radioactivity, some of which was TCA-precipitable and some nonprecipitable.

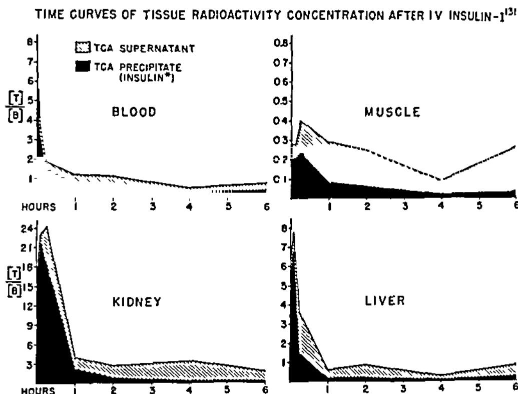


FIG. 6-5. Note the rapid increase in TCA supernatant radioactivity (degraded insulin-I^{131}) and the associated decrease in TCA-precipitable radioactivity (After Elgee, N J, Williams, R. H., and Lee, N. D.: *J. Clin Invest.* 33:1252, 1954.)

Newerly not only made similar observations with insulin but also with glucagon, γ-globulin, and albumin. Competitive inhibition of insulin binding was exhibited by these proteins. Insulin-I^{131} binds even more to diaphragm that has been boiled or treated with formaldehyde than to viable diaphragm. Cysteine-inactivated insulin showed the same degree of binding as untreated insulin.

It is possible that insulin can trigger certain biochemical reactions within a few seconds and then its presence is no longer necessary. It

has been shown in regard to the hepatic glycogenetic effect of hydrocortisone that most of the hormone in the liver disappears long before its maximal effects are accomplished.

A number of alterations in physiologic state have been shown by Elgee to influence the degradation and distribution of insulin-I^{131} in rats. Hepatectomy was associated with an increase in precipitable radioactivity in blood and skeletal muscle, there was not much effect on the renal concentration. Nephrectomy caused an increase in precipitable radioactivity in blood, muscle, and liver. After hepatectomy plus nephrectomy there was a marked increase in precipitable radioactivity in blood and muscle. With alloxan diabetes there was an increase in precipitable radioiodine in liver and blood. Triiodothyronine increased the degradation of insulin-I^{131} and led to a decrease in the amount concentrated in each tissue analyzed: liver, kidney, muscle, and blood. With thyroidectomy and hypophysectomy there was a decrease in insulin-I^{131} degradation. With each there was an increase in the concentration of TCA-precipitable radioactivity in the kidney and liver; after hypophysectomy there was also an increase in its concentration in the muscle and blood. With injection of insulin-I^{131} intraportally, there was no difference when compared with intracavally in the radioactivity in the blood and muscle; the concentration in the liver was greater after the intraportal injection and the concentration in the kidney was greater after the intracaval injection. Madison found in dogs that the endoportal administration of insulin caused the same degree of arterial hypoglycemia as the peripheral route of administration but a significantly reduced augmentation of peripheral glucose utilization, he assumed that an increased hepatic concentration of insulin decreased hepatic glucogenesis to a degree equivalent to the lesser stimulation of peripheral glucose uptake. Bell found that the injection in man of 1/80 unit of insulin per kg into an antecubital vein caused a *decrease* in femoral A-V glucose difference; the same amount injected into the right femoral artery produced an *increase* in A-V difference on the injected leg but a *decrease* in the opposite leg. These observations suggest that insulin can be rapidly fixed to tissue and directly stimulates an increase in glucose uptake by the tissue.

Wrenshall found that there was less insulin per pancreas in females than in males. The lowest concentration was in the head of the pancreas and the highest in the tail. About 1 per cent of islet weight was insulin. Normally, the pancreatic insulin concentration was proportionate to the surface area of the body and increased with age until the fifth decade. The quantity of insulin in the pancreas was very low with the growth-onset type of diabetes Although the quantity was not as little in

maturity-onset diabetes it still was only half of the control value for the fifth decade. It was very low in diabetic acidosis. A detailed discussion of the assays of insulin in the pancreas appears in Chapter 32.

SUMMARY

Understanding of the metabolism of insulin and glucagon should help clarify the pathogenesis of diabetes. Insulin-I^{131} and glucagon-I^{131} are rapidly distributed throughout the body. A considerable difference in the concentration in the tissues is found; kidney and liver accumulate the most, while erythrocytes and brain take out the least. There is evidence indicating that insulin penetrates cells, establishing a characteristic pattern of localization in intracellular components. It becomes fixed to tissues but is slowly released. Many factors influence the distribution of insulin and glucagon. With repeated insulin injections antibodies are formed that bind insulin, delaying its transfer to tissues and degradation. Juvenile diabetics have been found to have very little assayable insulin in the plasma and pancreas; maturity-onset diabetics have a lesser deficiency—indeed, some have been found to have a normal or hypernormal supply in the plasma. Insulin and glucagon are degraded in the body very rapidly, insulin probably by both enzymatic and nonenzymatic reduction as well as by proteolytic cleavage, and glucagon only by proteolysis. Factors influencing the degradation of these hormones, of course, may significantly influence their distribution and action.

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Chapter 7

THE "CELL MEMBRANE" THEORY OF THE ACTION OF INSULIN*

Rachmiel Levine and M. S. Goldstein

The postulate that insulin acts by increasing the transfer or transport of glucose from the extracellular fluid space into the cell interior was made in 1949 (22), on the basis of the following experimental observations:

In the eviscerated-nephrectomized dog, galactose is not metabolized to any significant degree. A load of galactose distributes itself in a "space" equal to about 40 to 45 per cent of the body weight. When insulin is given together with the galactose load, the volume of distribution of the sugar reaches the extent of total body water (about 70 per cent of body weight). These data were interpreted to mean that the membranes of certain cells were ordinarily virtually "impermeable" to galactose and that insulin, by acting on a transport system at the membrane, increased its activity. Because of the close chemical relationship between galactose and glucose it was assumed that the action of insulin on glucose was exerted in the same manner.

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In the same year Stadie (40, 41) demonstrated that insulin was "fixed" by the cell ("surface"?) and probably therefore exerted its effect in the "fixed" state. This work called attention to the need for a degree of intactness of the cell in demonstrating hormonal effects.

Subsequent work from our laboratory demonstrated that this action of insulin was restricted to a certain group of sugars, both hexoses and pentoses. This group is characterized by (1) having the "glucose" type of configuration at C_1, C_2, and C_3; and (2) an alcoholic OH group at C_6. The insulin responsive sugars were D-glucose, D-galactose, D-xylose and L-arabinose. The insulin nonresponsive group consisted of the keto sugars, D-mannose, D-glucuronic and D-galacturonic acids, D-arabinose, etc. (10, 23, 24, 25).

By direct carcass analyses it was also shown that the increased transport of such sugars as galactose, brought about by insulin, was not due to metabolic utilization, since the total injected amount was recovered.

These data were *confirmed and extended by many other* laboratories. Wick and Drury (43, 44) demonstrated the insulin effect on galactose transport in the eviscerated rabbit. Haft, Mirsky, and Perisutti (12a) and Resnick and Hechter (33) studied the uptake of sugars by rat diaphragm in vitro, and demonstrated the effect of insulin in hastening the *distribution of the nonutilizable galactose. Fisher (9) showed the effect* on galactose using the perfused rat heart, while Kruhoffer (15) employed the perfused hind limb of the dog.

A strong indication that insulin acted on the membrane transfer of glucose itself came from the work of Ross (35, 36, 37) on the entry of *sugars into the anterior chamber of the eye (in vivo) and into the* rabbit lens (in vitro). This investigator studied the transport of both nonutilizable and utilizable sugars in the normal and alloxanized rabbit.

Park and his group (28, 29, 30, 31) used conditions that depressed hexokinase activity, and demonstrated that under such circumstances *insulin could be shown to promote the transport of glucose into skeletal* and cardiac muscle. The level of intracellular free glucose was raised by insulin, showing that the action of the hormone was exerted on a reaction prior to glucose phosphorylation. These workers have also demonstrated that insulin affects the rate of sugar transport *into* and *out of* the cell. *The transport system showed stereospecificity; and competitive in*hibition could be demonstrated for the insulin-sensitive group of sugars.

It had been known for many years that in many tissues and organs the glucose utilization rate was not under the influence of insulin. The √ insulin *insensitive* tissues are: brain, kidney, gastrointestinal tract, the *red blood cells, etc. The following* tissues are insulin *sensitive:* skeletal muscle, cardiac muscle, fat cells, fibroblasts, leukocytes, the ciliary

body, and the optic lens. Yet specific sugar transport systems exist even in the insulin insensitive group (20, 34, 38). The liver seems to operate in a different manner; its cells are easily "permeable" to a large variety of all types of sugars (7). One is compelled, therefore, to conclude either that the transport systems of the insulin-sensitive tissues are different from those of the nonreactive cells, or that insulin exerts its action not on the transport system *proper* but on some molecular structure that usually "inhibits" or "covers" the transport portion of the cell membrane. We are inclined to the latter view because of the following experimental data:

1. If a tissue is injured, as occurs when a diaphragm is cut or kept in vitro for a prolonged period, the transport of sugars is enhanced, the insulin effect is diminished, but the stereospecificity persists.

2. Muscular work increases the rate of entry of both utilizable and nonutilizable sugars in the complete absence of insulin (11, 13, 17). We believe that this is due to a humoral factor liberated during exercise. The sugar specificity is the same as that for insulin.

3. Randle and Smith (32) have shown that oxygen lack, and drugs that inhibit oxidative processes, enhance glucose and xylose uptake by rat diaphragm in vitro.

The simplest hypothesis consonant with the data is to suppose that the sugar transport system is identical or closely similar in practically all tissues, but that the insulin-regulated cells possess in addition a "cover" for their transport areas. Insulin, in this view, reacts with the cover, perhaps causing it to retract, and thus exposes the transport system to the suitable substrate. That the transport system can be inhibited appears from the work of Park *et al.* (31) who found (using the perfused rat heart) that GH given for some days in vivo leads to a diminished transport rate, while hypophysectomy raises the rate of sugar entry. The effects of the adrenal cortex (24) and of GH addition in vitro (5), are not consistent in different laboratories.

The relationship of insulin action to the metabolism of the liver cell remains obscure. While there exists a body of evidence that under certain conditions, in some species, insulin may stimulate glucose uptake, glycogenesis, and lipogenesis in liver slices, and in the perfused organ (8, 12), the bulk of work in this field leads to the conclusion that no direct effect of insulin on liver is demonstrable (1, 26). For many years it was assumed that insulin inhibits hepatic sugar output. The most recent work throws doubt on this supposed action.

When C^{14} glucose is given to the intact animal, the specific activity of the blood glucose decreases in exponential fashion. This is due to the dilution effect of released hepatic glucose derived from nonisotopic

precursors. When glucose and/or insulin is given the decline in the specific activity is interrupted and a "plateau" in specific activity values sets in for a short time. After this period the exponential decrease in specific activity continues. The plateau has been interpreted as inhibition of liver sugar output (14, 18). This interpretation is thoroughly logical but was not tested experimentally. Recently, in collaboration with Dr. Robert Steele of the Brookhaven Laboratories, we have begun to examine the effects of glucose and insulin on the curve of C^{14} specific activity (42). It is already evident from this work that in the *liverless* animal one can get plateaus of specific activity, which are of course not due to inhibition of liver sugar output. The plateau is probably due to equilibration phenomena between pools of glucose. Certainly it cannot be claimed to denote unequivocally inhibition of liver sugar output.

For the time being the relation of liver to insulin action remains unresolved. We still hold to the view that insulin may not act directly on the liver cell.

From three laboratories comes recent evidence that insulin may promote the transport of many of the amino acids into muscle tissue in vitro (19, 32, 47). This action is observable in the absence of glucose in the medium, and thus seems to be exerted directly upon the cell mechanism for uptake of amino acids. This is consistent with the data of Ingle (16), in the eviscerated rat, but in contradiction to earlier data (21). The above work was done using C^{14}-labeled amino acids. Using nonisotopic amino acids in bulk, we could not observe a primary insulin effect on the rate of uptake into the tissues of the eviscerated nephrectomized dog.

Several recent observations are at present difficult to reconcile with the pure membrane theory.

Shaw and Stadie (39) found that insulin stimulated glycogen synthesis from C^{14} glucose but did not at the same time lead to increased lactic acid production. Two glycolytic pathways are postulated, only one of which is stimulated by insulin. This suggestion requires careful study, especially when one considers such data as those of Renold (45), who showed that in adipose tissue there was equivalent stimulation by insulin of sugar uptake, as well as of glycogenesis and CO_2 production.

Burk *et al.* (6, 46) find stimulation of glycolysis by insulin in brain and tumor homogenates under special conditions. This is inconsistent with observations in vivo that glucose uptake by brain is uninfluenced by insulin administration. The observation in vitro remains unexplained.

Bessman *et al.* (4) found that a diaphragm cut into small pieces admitted xylose freely, but an effect of insulin on glycogen deposition from glucose was still obtained.

Chain and co-workers (2, 3) could not obtain an insulin effect on galactose entry into the rat diaphragm. To our knowledge this is the only instance in which the galactose effect was not shown. They contend also that while insulin promotes glycogenesis it fails to increase the yield of CO_2. Glucose itself increases CO_2 production but not glycogenesis. Chain concludes that insulin must act on a pivotal reaction in cell energetics and not on membrane transport. The conclusions arrived at by Chain *et al.* seem too sweeping, considering the large number of workers who have provided evidence for transport of nonutilizable sugars. In addition, many of the conclusions are based upon counts of radioactivity in intermediates, without due consideration of their turnover.

The actual demonstration on a molecular level of the reaction of insulin with a portion of the sugar transport system has of course not been achieved and appears to be fraught with many technical difficulties. However the theory has much collateral evidence in its favor, and is capable of accounting for most of the metabolic effects of insulin in vivo and in vitro. It has focused attention on the specific cell surface mechanisms responsible for the regulation of substrate traffic and the profound effects some hormones may have by affecting the rates of exit and entry from and into cells.

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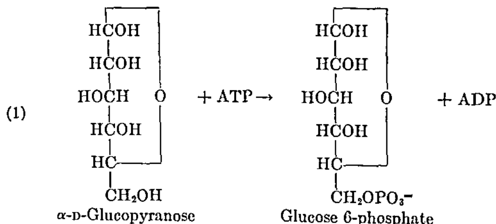
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Chapter 8

CARBOHYDRATE METABOLISM

DeWitt Stetten, Jr., and Glenn E. Mortimore

The metabolism of carbohydrates in animal tissues will be considered from the point of view of the sources and fates of glucose and its derivatives. Glucose, to be utilized, must first enter the cell (see Chap. 7). Within the cell, its preponderant chemical fate is phosphorylation at position 6 by adenosine triphosphate (ATP) in the presence of the ubiquitous hexokinase and Mg^{++}.



α-D-Glucopyranose   Glucose 6-phosphate

This important reaction is irreversible and exergonic, and, cell membranes being relatively impervious to phosphoric acid esters, the reaction serves to lock the sugar in the intracellular compartment. Numerous fates are available to glucose 6-phosphate and to many of its metabolic derivatives and attempts to represent all these reactions in a single diagram result in vast networks of limited usefulness. Furthermore, such diagrams give the erroneous impression that all the manifold reactions represented are occurring simultaneously and within a single cell or tissue. It is more convenient to dissect arbitrarily from this reticulum the most studied sequences or "pathways," since these are believed to include the most important reactions.

ANAEROBIC GLYCOLYSIS

The familiar *glycolytic* sequence is represented in Figure 8-1. Commencing with glucose and terminating with lactic acid, no net oxidation is involved and indeed this sequence proceeds readily anaerobically. Reaction 6 involves reduction of diphosphopyridine nucleotide (oxi-

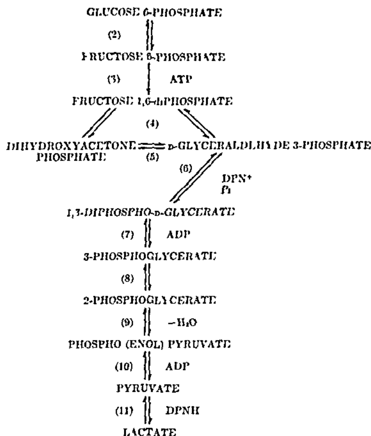


FIG. 8-1. Pathway of anaerobic glycolysis.

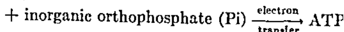
dized (DPN⁺), but the diphosphopyridine nucleotide (reduced) (DPNH) formed is again oxidized at step 11. The enzymes concerned are all in solution in the intracellular water. ATP is expended not only in the generation of glucose 6-phosphate, but also in the formation of fructose diphosphate. ATP is recovered in the decomposition of diphosphoglycerate and again from phospho(enol)pyruvate, with a net yield of 2 moles of ATP per mole of glucose glycolyzed. If pyruvate is considered the terminus of glycolysis, an additional 6 moles of ATP from the oxidation of DPNH generated at step 6, or a total of 8 moles, will arise. The nature of this so-called "oxidative" phosphorylation will be considered below. In skeletal muscle, when operating under circumstances of limited oxygen supply, it is this sequence from glucose or glycogen to lactic acid that supplies most of the energy, and it is the accumulated lactic acid that accounts for the well-known oxygen debt. Alcoholic fermentation involves the same reactions as those of anaerobic glycolysis as far as pyruvic acid. The former process terminates with the production of ethanol; the latter with lactic acid.

TRICARBOXYLIC ACID CYCLE

Aerobically, pyruvate may undergo oxidative decarboxylation to acetyl coenzyme A (acetyl CoA). Alternatively, by one of several routes, pyruvate may combine with CO_2 to yield oxaloacetic acid. Thus pyruvate may serve as precursor to both of the necessary ingredients for the tricarboxylic acid cycle (Fig. 8-2). This sequence stands in contrast to the glycolytic sequence in several regards. Reactions 12, 16, 17, 18, and 20 involve oxidation of substrates. Happily, the enzymes of the tricarboxylic acid cycle are situated within the mitochondria, which also house the oxidoreduction enzymes of the electron transport system, flavoproteins—cytochromes—cytochrome oxidase. The tricarboxylic acid cycle also is a major source of CO_2, evolved at steps 12, 16, and 17, whereas the glycolysis of glucose to lactic or pyruvic acid yields no CO_2.

Oxidations of these or other substrates in the body are best viewed as transfers of electrons from initially high energy states to successively lower energy levels, ultimately to the lowest available energy level of water. A portion of the energy released as these electrons cascade downward is recovered as energy-rich compounds of phosphate, such as ATP, the endergonic synthesis of which is coupled with the exergonic oxidative process:

adenosine diphosphate (ADP)



$$+ \text{ inorganic orthophosphate (Pi)} \xrightarrow[\text{transfer}]{\text{electron}} \text{ATP}$$

When DPNH is oxidized by mitochondria, a maximum of three moles of ATP are synthesized per atom of oxygen consumed and this ratio is believed to obtain at reactions 12, 16, and 20. In addition, at step 17 the initial product is succinyl CoA, which, in the course of its conversion to succinic acid, yields an additional mole of ATP, or a total of

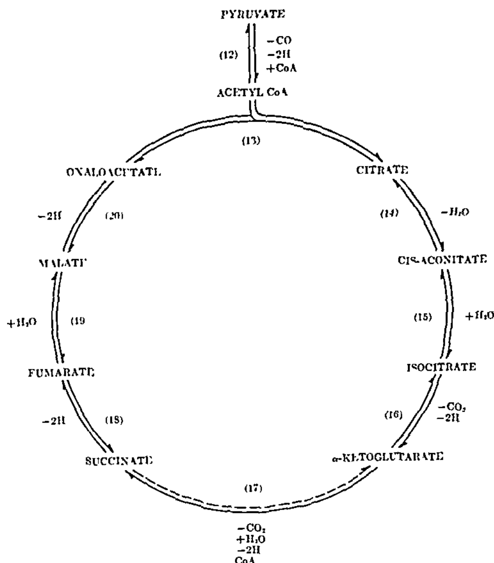
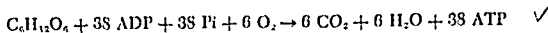


FIG. 8-2. Tricarboxylic acid cycle.

four for the entire step. Step 18 involves succinic dehydrogenase, an enzyme not utilizing DPN at all, and undoubtedly related to this fact, it appears to generate only two moles of ATP. The total yield of ATP anticipated per revolution of the cycle is fifteen moles. Since two moles of pyruvate result from the glycolysis of each mole of glucose, giving

eight moles of ATP, the complete oxidation of glucose via glycolytic and tricarboxylic acid pathways may be expected to yield 8 + 2(15) = 38 moles of ATP.



$$C_6H_{12}O_6 + 38\ ADP + 38\ Pi + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP$$

The above equation is designed to exhibit the important association between glucose catabolism and "phosphorylation," meaning in this instance the generation of high energy compounds of phosphate at the expense of inorganic phosphate. This class of compounds, of which ATP is the most prominent, but which includes all the polyphosphates of nucleotides (e.g., uridine triphosphate (UTP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), etc.), creatine phosphate, and others, serves as the immediate energy source for most of the endergonic processes *in the body. Among these processes may* be listed muscle contraction, nerve conduction, secretion or active reabsorption and synthesis of most macromolecules. In this sense, the generation of ATP is a very important aspect of glucose catabolism.

The biochemist distinguishes between substrate phosphorylation (e.g., reactions 7, 10, and 17) and oxidative phosphorylation. The former class comprises those reactions in which inorganic phosphate forms a known compound with a substrate, which then undergoes chemical change. The phosphate is subsequently surrendered to ADP to yield ATP. Reactions of oxidative phosphorylation cannot today be precisely described. Inorganic phosphate and ADP are consumed, ATP is formed, during the transfer of electrons through the pyridine nucleotide—flavoprotein—cytochrome—cytochrome oxidase sequence. Although not *susceptible of precise definition, oxidative phosphorylation is readily* identified by the experimental procedure of dinitrophenol poisoning. This reagent and certain others break the loose coupling between oxidation and phosphorylation, permitting the former to occur in absence of the latter process.

PASTEUR EFFECT

The dependence of both the glycolytic and tricarboxylic acid sequences upon a supply of inorganic phosphate and ADP is probably an important factor in the Pasteur effect. This effect may be described as the suppression of glycolysis by the presence of oxygen. In many systems glycolysis proceeds more rapidly in the absence of oxygen than it does aerobically. All the Pi and ADP within the cell, anaerobically, is *available for those steps of glycolysis* which require these reagents (6, 7, and 10). When oxygen is admitted, a competition is established for Pi and ADP between the substrate-coupled processes in glycolysis and

the oxidative processes of the tricarboxylic acid cycle. This competition results in a decreased glycolytic rate.

The Pasteur effect is an internal chemoregulatory system that acts to conserve glucose. When total oxidation of glucose is possible, a smaller amount of glucose is needed to supply the energy requirement than when anaerobic glycolysis alone is proceeding. It is noteworthy that when agents which uncouple oxidative phosphorylation, e.g., dinitrophenol, are added to such a system, the Pasteur effect is abolished and glycolysis is uninfluenced by availability of oxygen. Insofar as thyroxin may share this effect of dinitrophenol, this abolition of the Pasteur effect may explain in part the metabolic consequences of thyrotoxicosis.

PENTOSE PHOSPHATE PATHWAY

The glycolytic sequence is but one of several fates of glucose 6-phosphate. In certain tissues such as liver and mammary gland, glucose 6-phosphate may undergo oxidation to 6-phosphogluconic acid, which in turn may be oxidatively decarboxylated to yield ribulose 5-phosphate (Fig. 8-3). The sequence of reactions thus initiated has been variously termed the hexose monophosphate shunt, the 6-phosphogluconate oxidation pathway, or the pentose phosphate pathway. It differs from glycolysis in several major regards. The oxidative steps characteristically involve, not DPN^+, but rather triphosphopyridine nucleotide (oxidized) (TPN^+), and result in the formation of triphosphopyridine nucleotide (reduced) (TPNH). This reduced cofactor is produced in a relatively limited number of reactions and is specifically needed in the synthesis of fatty acids from acetoacetyl CoA (see Chap. 9). The accumulation of acetoacetic acid and the other ketone bodies, and the suppression of fatty acid synthesis in uncontrolled diabetes, have been attributed to inadequacy of TPNH production owing to failure of glucose 6-phosphate oxidation.

The oxidative pathway differs from glycolysis in other regards also. Carbon dioxide is produced, oxygen is consumed, early in the reaction sequence. ATP is not required for the oxidation of glucose 6-phosphate, whereas it is required for its glycolysis. The remaining reactions that have been included in this pathway are represented in Figure 8-3. It will be seen to resemble a maze rather than a simple pathway. Note the multiplicity of function of the enzyme, transketolase, which, in the presence of thiamine pyrophosphate, transfers the ketol (CH_2OH—CO—) grouping from various donors to various acceptors.

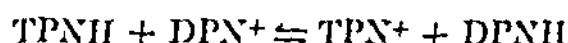
The enzyme transaldolase in a similar fashion transfers the three-carbon fragment (CH_2OH—CO—CHOH—) from one sugar to another.

FIG. 8-3. Pentose phosphate pathway.

By the judicious combination of these reactions with a portion of the glycolytic sequence, one may reconstruct a scheme that will effect the total oxidation of glucose to CO_2.

A variety of interesting sugar derivatives are generated in the 6-phosphogluconate oxidation pathway. Among these is the seven carbon ketose, sedoheptulose 7-phosphate, as well as a number of pentose derivatives. The ribose phosphate formed may serve as a source of this

sugar in ribonucleic acid synthesis. The relationships of these pentoses to others will be considered subsequently. The TPNH formed in th initial oxidative reactions may in part be oxidized over the flavoprotei —cytochrome—cytochrome oxidase sequence. In contrast to the well established coupling of phosphorylation to oxidation of DPNH, couple phosphorylation has not been observed when TPNH is serving as sub strate. However, by demonstration a transhydrogenase:



$$TPNH + DPN^{+} \leftrightarrows TPN^{+} + DPNH$$

which in effect would replace the one reduced pyridine nucleotide b the other, makes such a coupling with TPNH conceivable. It is of in terest that this transhydrogenase reaction is held to be sensitive t estrogen in vitro.

GLYCOGENESIS AND GLYCOGENOLYSIS

Since the ingestion of dietary carbohydrate is discontinuous, it i convenient that a storage form of glucose is provided in most tissues Except in certain tumor cells, glycogen seems to be a characteristic mammalian cell constituent. This polysaccharide is made up of many glucose residues linked to each other by α-1 → 4 and α-1 → 6 glucosidic bonds, in which the former predominate. A treelike branching pattern has been established and molecular weights as high as 200,000,000 have been recorded. Glycogen synthesis stems from glucose 1-phosphate, which reacts as follows in the presence of a polysaccharide and phosphorylase.



$$\text{Glucose 1-phosphate} + (\text{glucose})_{n} \rightleftharpoons (\text{glucose})_{n+1} + \text{Pi}$$

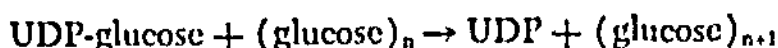
The bonds formed or phosphorylized by this reversible reaction are always of the α-1 → 4 variety.

Phosphorylase of liver and of muscle have been intensively studied. The liver enzyme is itself enzymatically inactivated and its reactivation requires, in addition to a specific activating enzyme, ATP and an anhydride of adenosine monophosphate (AMP). The last named cofactor is itself generated from ATP in the presence of epinephrine or glucagon and it is believed that by this mechanism epinephrine and glucagon maintain hepatic phosphorylase activity. An inactive phosphorylase has been studied in muscle. This material can be activated by addition of AMP. Epinephrine, but not glucagon, apparently favors the activation of muscle phosphorylase.

The establishment of α-1 → 6 bonds in glycogen is due to a branching enzyme, a transglycosidase that breaks an α-1 → 4 bond to create an

α-1 → 6 bond. The rupture of α-1 → 6 bonds is effected hydrolytically by a specific debranching enzyme, α-1 → 6 amyloglucosidase. Rare types of glycogen storage diseases have been attributed to abnormalities in these enzymes. The more common von Gierke's disease is due to a lack of glucose 6-phosphatase in liver and kidney, with consequent damming up of hexose monophosphates and displacement of the equilibrium of the phosphorylase reaction toward synthesis. Since this enzyme is normally absent from muscle, no comparable changes are seen in this tissue.

Recently a second synthetic route to glycogen has been indicated in liver:

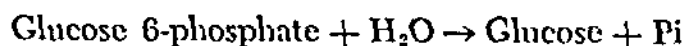


$$\text{UDP-glucose} + (\text{glucose})_n \rightarrow \text{UDP} + (\text{glucose})_{n+1}$$

This reaction resembles the phosphorylase reaction in its requirement for a pre-existing seed of polysaccharide and is a model for the synthesis of various other polysaccharides, including chitin. For a further discussion of the biochemistry of mucopolysaccharides, the reader is referred to Chapter 13.

GLUCONEOGENESIS

The glucose of the body fluids arises essentially from two sources, the portal absorption of the products in intestinal digestion and the hydrolysis, primarily hepatic, of glucose 6-phosphate. The former of these obviously fails in starvation and may also be subnormal owing to failure of digestion, as in pancreatic insufficiency, or failure of absorption, as in sprue. Except for the small component of glucose liberated by the attack of debranching enzyme on glycogen (see p. 96), all the remaining glucose is derived from the reaction:



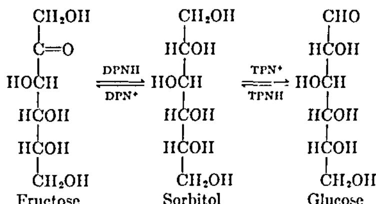
$$\text{Glucose 6-phosphate} + H_2O \rightarrow \text{Glucose} + \text{Pi}$$

The enzyme that mediates this hydrolysis is a specific glucose 6-phosphatase that appears to be lacking in muscle but is abundant in liver, kidney, and probably in other tissues. Any compound capable of giving rise to glucose 6-phosphate in liver is therefore potentially glucogenic. This includes all the products derived from glucose (see Figs. 8-1, 8-2, 8-3) and also all substances that can generate any of these compounds. The carbon skeletons of many of the amino acids can be transformed into the α-ketoacids, oxaloacetic, pyruvic, α-ketoglutaric (see Chap. 10). Glycerol from fat serves as a potential source of triose phosphate. These and other noncarbohydrate precursors of glucose are called gluconeogenic substances. The hyperglucemia that follows administration of

glucocorticoids is undoubtedly in part due to enhanced gluconeogenic activity.

The mammalian body is equipped to handle a number of sugars other than glucose. This it does, in certain cases, by rearrangement of the molecule into the glucose configuration. The enzymes involved appear to be concentrated in the liver, which is an ideal arrangement since sugars such as fructose and galactose enter the body by the portal route.

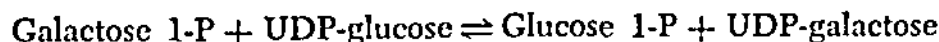
Fructose may be phosphorylated in position 1 by ATP and a fructokinase to yield fructose 1-phosphate. By the action of an aldolase, this is split to yield dihydroxyacetone phosphate and glyceraldehyde, both glucogenic materials. Alternatively, fructose may be reduced to sorbitol, which, upon reoxidation, may yield glucose:



By this series of reactions either fructose or sorbitol, both of which have had their advocates in the treatment of diabetes, may assist in the formation of TPNH (see p 94).

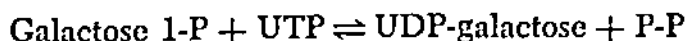
SOME DEFECTS IN CARBOHYDRATE METABOLISM

The interrelations between galactose and glucose are shown in Figure 8-4. *Galactose 1-phosphate* is formed in liver by the action of galactokinase, and is next transformed into UDP-galactose by reaction with either UDP-glucose,



$$\text{Galactose 1-P} + \text{UDP-glucose} \rightleftharpoons \text{Glucose 1-P} + \text{UDP-galactose}$$

or with UTP,



$$\text{Galactose 1-P} + \text{UTP} \rightleftharpoons \text{UDP-galactose} + \text{P-P}$$

The latter mechanism appears to be deficient in infancy and the former is the process specifically nonoperative in galactosemia. The galactosemic infant thus has no route of disposal of galactose 1-phosphate available to him. An epimerization about carbon-4 of the sugar effects interconversion of UDP-galactose and UDP-glucose.

GALACTOSE

ATP Mg^{++}

GALACTOSE 1-P

UDP GALACTOSE

BLOCKED IN GALACTOSEMIA

GLUCOSE

BLOCKED IN SOME GLYCOGENOSES

UDPG

UDPGal

GLUCOSE 6-P

GLUCOSE 1-P

UTP

PP

UDP GLUCOSE

2DPN⁺

H_2O

UDP GLUCURONIC ACID

BILIRUBIN CONJUGATION BLOCKED IN CONSTITUTIONAL HEPATIC DYSFUNCTION

GLUCURONIC ACID CONJUGATION
MENTHOL
SALICYLIC ACID
STEROIDS
BILIRUBIN ETC

D-GLUCURONIC ACID

GLUCOSE

GLYCOGEN

TPNH

D-XYLULOSE 5-P

ATP

D-XYLULOSE

DPN⁺ −2H

XYLITOL

TPNH

BLOCKED IN PENTOSURIA

L-XYLULOSE

DPN⁺ $-CO_2$ −2H

L-GULONIC ACID

BLOCKED IN MAN PRIMATES GUINEA PIGS

L-ASCORBIC ACID

Fig. 8-1. Some defects in carbohydrate metabolism.

UDP-glucose also serves as the immediate precursor of glucuronides. The sugar moiety may be oxidized by DPN^+ to UDP glucuronic acid, whence the glucuronic acid residue may be transferred by a microsomal enzyme to such acceptors as menthol, aromatic amines, certain steroids, bile pigments, etc. This latter step is blocked in constitutional hepatic dysfunction.

Free glucuronic acid (Fig. 8-4) gives rise to L-gulonic acid, which, in species other than man, primates, and the guinea pig may be further oxidized to ascorbic acid. The absence of the requisite enzyme in the above named species makes these dependent upon a dietary supply of vitamin C. Alternatively, L-gulonic acid may be decarboxylated to L-xylulose, which is normally transformed, via xylitol, into D-xylulose, a glucogenic material. In the patient with congenital pentosuria, the enzyme TPN-xylitol dehydrogenase (Fig. 8-4) is lacking, leading to the urinary excretion of accumulated L-xylulose.

Whereas a number of the defects in carbohydrate metabolism, e.g., galactosemia, von Gierke's disease, pentosuria, etc., can be pinpointed biochemically and attributed to the lack of a specific enzyme, such is not the case with diabetes. Of the manifold consequences of relative or absolute insulin deficiency, several seem to relate to defects in carbohydrate metabolism, but these are not susceptible to sharp definition.

The ability of muscle to remove glucose from its environment in the diabetic animal is severely impaired. This defect is readily demonstrable in the isolated diaphragm and may be reversed by the *in vitro* addition of insulin. The well-known reduced lactate production after glucose administration to the diabetic is also attributable to this impairment in glucose removal by muscle, as is the failure of the respiratory quotient to approach unity during glucose administration. There is a considerable body of evidence to suggest that in some fashion insulin facilitates the entry of blood glucose into the intracellular compartment of muscle (see Chap. 7). On the other hand, there is evidence to suggest that insulin may affect the activity of intracellular enzymes, specifically hexokinase (see Chap. 14).

The situation with respect to liver is less well delineated. Insulin deficiency results in enhanced activity of hepatic glucose 6-phosphatase, thereby providing for the rapid hydrolysis of glucose 6-phosphate and increased rate of hepatic glucose production. This abnormality has not been corrected in isolated liver preparations. Insulin, however, will reduce the activity of this enzyme when administered in vivo, but only after a considerable lapse of time. By way of contrast, administration of insulin to rats is promptly followed by an increase in the capacity of the muscles to take up glucose from the blood.

The conclusion based upon such experiments, as well as upon experiments that failed to demonstrate changes in transhepatic A-V difference in glucose concentration after insulin injection, is that the immediate target organ for insulin is the muscle. This conclusion is in disagreement with that derived from isotope studies in dog and in man that revealed an immediate cessation of glucose release, presumably by liver, after insulin administration. The resolution of this apparent paradox must await further experiments.

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Chapter 9

THE LIPID DERANGEMENTS OF DIABETES

Marvin D. Siperstein

While the most striking metabolic lesion that accompanies the diabetic state is, of course, the reduced ability to catabolize glucose, a number of other biochemical abnormalities of carbohydrate as well as of protein and lipid metabolism are regularly observed in both clinical and experimental diabetes. Of these, no doubt the most serious are the derangements in lipid metabolism. The accumulation of the ketone bodies, acetoacetic acid, β-hydroxybutyric acid and acetone, is probably responsible for the most severe symptoms seen in the uncontrolled diabetic. Coupled with this abnormality in lipid metabolism is an almost complete loss of the ability to synthesize fatty acids while at the same time there is an increased dependence on the breakdown of fatty acids to satisfy the energy requirements of the body. Finally, there is good evidence to indicate that the synthesis of another lipid, cholesterol, may be abnormally increased in the diabetic state, a fact that has been invoked to explain the high incidence of atherosclerosis in this disease.

These abnormalities in lipid metabolism together with those in other areas of intermediary metabolism make it apparent that diabetes is characterized by not just one but by a spectrum of biochemical derangements involving numerous aspects of cellular metabolism. Despite

this diversity of metabolic lesions, evidence is accumulating which suggests that many of these abnormalities are in fact closely interrelated. It will be the aim of this chapter, therefore, to attempt both to summarize some of the newer advances in our understanding of lipid metabolism and to determine how these recent findings help to clarify the nature of the various lipid abnormalities found in the diabetic state.

LIPOGENESIS

Quantitative Significance

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The physiologic importance of this conversion of carbohydrate to fat in the normal state of course depends on its quantitative significance under conditions of normal caloric intake, especially in a nongrowing animal. Stetten and Boxer (33) in 1944, using deuterium-labeled water, calculated that under the above conditions an adult rat will store only

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3 per cent of ingested glucose as liver and muscle glycogen. On the other hand, it was estimated from the turnover time of total body fat that 30 per cent of ingested glucose will be utilized to produce fatty acids. It is apparent, therefore, that *of the glucose carbons stored in the body*, some 90 per cent are retained in the form of fat.* Other calculations of the turnover time of total body fat ranging from 7 days for mice to from 13 to 25 days for rats have been reported, and all would *indicate that animals must convert nonlipid materials, i.e.*, primarily glucose, into fatty acids at very significant rates.

It may be considered as proved, therefore, that under normal circumstances, lipogenesis must be a quantitatively important process and that the *conversion of glucose to fatty acids does in fact represent the* major mechanism by which the body is able to store the energy of glucose.

Site of Conversion of Glucose to Fatty Acid

Following the demonstration that fatty acids could be synthesized by the intact animal, a great deal of evidence was accumulated to indicate that the liver was the major site of lipogenesis in the body. It is possible, however, to find indications even in the early experiments that this conclusion was incorrect. A comparison of the turnover rates of liver fat with that of total body fat suggests that the liver might not be of great importance in fat synthesis. Stetten calculated that 1.9 Gm. of fat was being synthesized per day by the rat (33). If a normal rat liver contains approximately 200 mg. of fat, of which 40 per cent or 80 mg. is being replaced each day (33), then only 4 per cent ($^{80}/_{1900} \times 100$) of the total body fat could possibly be contributed by hepatic lipogenesis.

The best evidence that *extrahepatic* tissue does play the dominant *role in lipogenesis was provided by the experiments of* Masoro and Chaikoff in 1949 (22). These investigators injected C^{14}-labeled glucose into intact rats and demonstrated that less than 5 per cent of the fatty acid synthesized from this glucose could be found in the liver in 8 hours. In view of the fact that the turnover time of liver fat is at least one day, transport of newly synthesized fat from liver to extrahepatic depots could not have accounted for these results. Furthermore, hepatectomized rats were capable of carrying out fatty acid synthesis from glucose as well as could the normal rats. These data provide strong evidence for the conclusion that the liver plays a quantitatively minor role in fatty acid synthesis.

* There is, however, no known mechanism by which this fat can actually be converted back into glucose

Largely through the studies of Shapiro and Wertheimer (29), Feller (10), and Hausberger (15), the problem of the extrahepatic site of lipogenesis has been resolved. It is now clear that most of the fat of the body is synthesized in the adipose tissue.

Sections of adipose tissue can, per gram of tissue, synthesize fat from either labeled acetate or glucose at a rate faster than that of liver (10, 15). If one considers adipose tissue as a single organ, its weight in a normal man is approximately 7 kg., and in a woman, approximately 15 kg., or between 5 and 10 times the normal weight of the liver. This fact coupled with the relatively enormous ability of adipose tissue to synthesize fat suggests that adipose tissue can account for the preponderance of extrahepatic lipogenesis. Some direct evidence in support of this conclusion has been provided by the studies of Faverger (9) in which attempts were made to compare the fatty acids synthesized in liver with that synthesized in adipose tissue by intact animals given acetate-C^{14} or glucose-C^{14}. It can be concluded from the figures that Faverger has published that adipose tissue in the *intact* organism incorporates glucose-C^{14} into fatty acids at a sufficiently rapid rate to account for the majority of lipogenesis in the whole animal.

The evidence now available therefore strongly supports the conclusion that, although the liver may play a role in the synthesis of fatty acids, the conversion of glucose to fatty acids takes place primarily in the adipose tissue of the body.

Biochemical Mechanism of Fatty Acid Synthesis

Much of our lack of understanding of the lipid lesions of diabetes exists because many of the important details of fat synthesis have not, as yet, been elucidated. The major biochemical steps of lipogenesis, according to present concepts, and their relation to cholesterol and ketone body synthesis are shown in Figure 9-1 (28). With a few minor exceptions all fatty acids synthesized within the cell are known to derive their carbon from the common catabolite of foodstuffs, acetyl CoA, i.e., the two-carbon compound, acetic acid, combined with coenzyme A. The initial reaction of fatty acid synthesis consists of the condensation of two molecules of acetyl CoA to form one of acetoacetyl CoA (Reaction 1 in Fig. 9-1). Acetoacetyl CoA is next reduced by diphosphopyridine nucleotide-H_2 (DPNH) to yield β-hydroxybutyryl CoA (Reaction 2). By dehydration of the latter compound through the action of the enzyme, hydrase, a molecule of crotonyl CoA is formed (Reaction 3), and finally crotonyl CoA is reduced to yield the first true activated fatty acid, butyryl CoA (Reaction 4). Since the latter may well be the limiting reaction of lipogenesis, it should be emphasized that reduced

*tri*phosphopyridine nucleotide (TPNH) serves as the hydrogen donor in the reduction of crotonyl CoA (18). Another molecule of acetyl CoA can now condense with the 4 carbons of butyryl CoA to yield the forerunner of a 6-carbon fatty acid. By successive condensations with acetyl CoA and reductions by DPNH and TPNH, a long chain activated fatty acid such as palmityl CoA is finally produced. The free fatty acid then can be formed by hydrolysis. On the other hand, the activated fatty acids may combine with glycerol to form triglycerides, or with cholesterol to yield cholesterol esters.

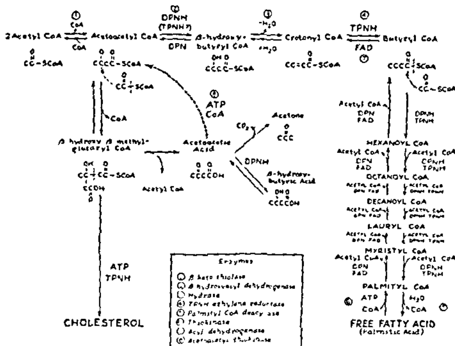


FIG. 9-1. Details of fatty acid synthesis and breakdown and their relationship to cholesterol and ketone body synthesis.

Recently there has been described another pathway of fatty acid synthesis, which utilizes two molecules of reduced triphosphopyridine nucleotide as the reducing agent for each acetyl CoA added to the fatty acid chain. In addition, adenosine triphosphate, biotin, and carbon dioxide are required in this series of reactions; however, the role of these substances in this pathway of lipogenesis is not yet known (37).

The Diabetic Defect in Lipogenesis

There is now abundant evidence that diabetes results in a marked decrease in the ability to carry out the synthesis of fatty acids as described in the preceding section. In view of the evidence presented

earlier that lipogenesis is the chief means of storing carbohydrate, it is obvious that such a metabolic defect must have serious effects on the body economy. The consequences of this lesion have been most dramatically shown by the experiment of Drury (8) in which diabetic rats or dogs subjected to alternate days of fasting and of feeding suffered marked weight loss in the course of only one week of such treatment. Normal animals maintained their body weights on this program; likewise, diabetic rats when fed daily did not lose weight. In retrospect it is clear, as Drury concluded, that the inability of these animals to store carbohydrate, even for a period of one day, is the direct result of their inability to synthesize fatty acids, and it is therefore apparent that under these circumstances lipogenesis is a function without which the diabetic animal cannot survive.

Convincing evidence of the inability of the diabetic to synthesize fat was first obtained by Stetten and Boxer when they demonstrated that the incorporation of deuterium-labeled water into fatty acids was greatly depressed in the intact diabetic rat (34). This finding was readily confirmed with C^{14}-labeled glucose both in isolated liver slices of alloxan diabetic rats and in intact animals (4).

Subsequently the cause of the diabetic defect in lipogenesis has been the subject of a great deal of study. This lesion could be due to metabolic blocks at any of the numerous biochemical steps involved in glycolysis and lipogenesis. In fact, the known site of insulin action at the entrance of glucose into the cell or in its conversion to glucose-6-phosphate should of itself result in a decrease in fat synthesis from glucose. It should be noted, however, that the diabetic block in lipogenesis has been shown to be much more severe than the block in glucose oxidation, a finding which suggests that the latter defect is not the direct and only cause of the inability to convert glucose to fatty acid. The studies of Chernick and Chaikoff have, furthermore, clearly demonstrated that in diabetic liver, the synthesis of fat from fructose-C^{14} and acetate-C^{14}, as well as from glucose-C^{14}, is greatly depressed. As summarized in Figure 9-2, this finding indicates that at least two blocks must be invoked to account for the diabetic defect in the conversion of glucose into fat, one at the point of glucose phosphorylation and the second on the pathway of incorporation of two carbon units into fatty acids.

The next question that had to be answered was whether insulin itself has two sites of action or whether in some manner the second, i.e., the lipogenic block in diabetes, is a consequence of the depressed breakdown of glucose that accompanies the lack of insulin. The latter explanation has proved to be the case. Baker *et al.* were able to show that

the synthesis of fatty acids from acetate could be repaired in the diabetic simply by feeding fructose, a sugar that does not require insulin for its utilization (3). From this experiment it seems likely that some factor produced during glucose breakdown is required for normal lipogenesis, and that in the diabetic state the depressed breakdown of glucose limits the supply of this factor and hence causes a depression in fat synthesis. In other words, the cell would appear to possess a means of stimulating fat synthesis when glucose breakdown is adequate and of decreasing lipogenesis when, for any reason, e.g., the diabetic state, the supply of glucose is decreased. As is discussed below, the mediator of this stimulation of lipogenesis is probably reduced TPN.

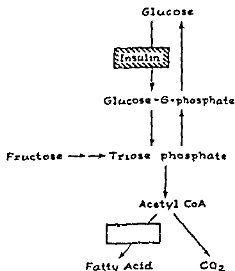


FIG. 9-2. Two metabolic blocks in diabetic liver.

Glucose breakdown, as discussed in detail in Chapter 8, takes place chiefly over the Embden-Meyerhof pathway; however, in a number of tissues, especially in those involved in fatty acid synthesis, e.g., adipose tissue, mammary gland, and liver, a significant fraction of the glucose uses an alternate route known as the pentose phosphate or hexosemonophosphate pathway (38). No TPNH is produced during Embden-Meyerhof glycolysis; in contrast, however, two molecules of TPNH are synthesized for each molecule of glucose using the pentose phosphate route. Studies in isolated liver preparations have indicated that the relatively small amounts of glucose utilizing the pentose phosphate route are responsible for the stimulation of lipogenesis by glucose oxidation (19, 30) and conversely the diabetic defect in lipogenesis is due

to a lack of the TPNH produced during glucose breakdown over the pentose phosphate route. This conclusion is supported by the fact that substitution of another source of TPNH will, to a large extent, repair the lipogenic defect in the diabetic liver even in the absence of glycolysis (31). The observation that a lack of TPNH is probably responsible for the failure of the diabetic to synthesize fat suggests, as is shown in Figure 9-3, that the exact location of the lipogenic block is at the site of conversion of crotonyl CoA to butyryl CoA. It should be pointed out, however, that in the pathway of lipogenesis which utilizes TPNH as the sole reducing agent (37) such a block would probably be located in the conversion of acetoacetyl CoA to β-hydroxybutyryl CoA.

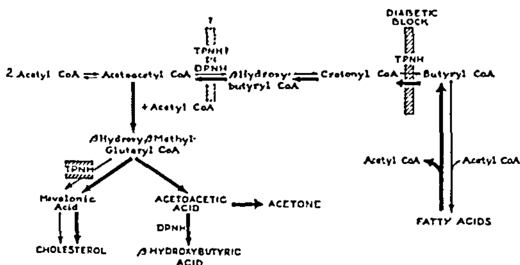


FIG. 9-3. Diabetic derangements in lipid metabolism.

Though most of the above studies have been carried out in liver, as noted earlier, the major site of lipogenesis under normal circumstances is probably not the liver but the adipose tissue of the body. It is therefore important to emphasize that lipogenesis is defective in the *intact* diabetic animal and thus it is obvious that adipose tissue must also be affected by the lipogenic lesion. Direct evidence that this is so has been provided by recent studies of Hausberger, which have demonstrated that a lipogenic defect is present in isolated adipose tissue of diabetic rats and the lesion is correctable with insulin (15). Furthermore, the concept that the extent of pentose phosphate oxidation may be of paramount importance in controlling lipogenesis in adipose tissue is supported by Renold's finding that insulin, added to adipose tissue, may produce a disproportionate stimulation in pentose phosphate oxidation (25).

triglyceride level are also observed in uncontrolled diabetes suggesting that several forms of lipid can be mobilized in response to the metabolic demands of the diabetic animal.

It must be emphasized, however, that the oxidation of *free* fatty acids must in some unknown manner be directly effected by glucose breakdown since unesterified palmitic acid is oxidized at an increased rate by diabetic rat liver slices, an abnormality also corrected by insulin pretreatment (20).

CHOLESTEROL SYNTHESIS

The fact that the body can synthesize cholesterol was well established by the careful balance studies of Schoenheimer and Breusch (26) and

Fig 9-4. Details of cholesterol synthesis

was amply confirmed by studying the incorporation of deuterium and carbon-labeled intermediates into cholesterol. Every organ of the body with the possible exception of the adult brain seems capable of carrying out this process; however, there is reason to believe that blood cholesterol is synthesized principally by the liver (13).

Though it was established over 10 years ago that acetate is the principal substrate from which cholesterol is synthesized, the details of the biochemical mechanism by which this two-carbon compound is built into the sterol molecule are only now beginning to be elucidated.

As will quickly be apparent from the relationships shown in Figure 9-1, our present knowledge of cholesterol synthesis makes it impossible to dissociate this process from those of lipogenesis and ketosis. A summary of the probable steps involved in cholesterogenesis is shown in Figure 9-4. As in lipogenesis, the initial reaction in the synthesis of cholesterol consists of the formation of acetoacetyl CoA by the condensation of two molecules of acetyl CoA. At this point the two pathways diverge in that sterol synthesis here requires that a third acetyl CoA be added to the acetoacetyl-CoA to yield a six carbon compound, β-hydroxy-β-methyl glutaryl CoA (HMG CoA). It is from this intermediate that both cholesterol and the ketone bodies (2) are derived. HMG-CoA next loses its CoA and is reduced by two molecules of TPNH to yield the six-carbon compound known as mevalonic acid (35), as shown in Figure 9-4. The aldehyde, mevaldic acid, is a possible intermediate in this reaction. After activation by two molecules of ATP, ✓ the mevalonic acid combines with two similar compounds and following loss of three carbon dioxide molecules produces the fifteen-carbon precursor of cholesterol, farnesenic acid (7). Two of these molecules next combine to give squalene, which in Figure 9-4 is drawn to conform to the structure of a sterol molecule. The cyclization of squalene, which is initiated by TPNH and oxygen, then yields lanosterol, the first true sterol in this series of reactions (36). Finally, lanosterol again through the action of TPNH and oxygen undergoes a loss of three carbons as carbon dioxide and, after the necessary shift of one double bond and the reduction of the other, is converted into cholesterol (23).

Cholesterol Synthesis in Diabetes

While it has been clearly shown with C^{14}-labeled acetate that the livers of diabetic rats can synthesize cholesterol at increased rates (16), there is some difference of opinion as to whether all diabetic animals synthesize an excess of cholesterol. Some investigators have observed a normal rate of cholesterol synthesis and others a decreased synthesis in severely diabetic rats, and it seems probable, therefore, that cholesterolgenesis varies somewhat with the degree of severity of the disease.

The mechanism by which diabetes may produce these effects on cholesterol synthesis is not known; however, one possible explanation becomes apparent from the relationships shown in Figure 9-3. A relative deficiency of TPNH, as noted, is probably responsible for the diabetic defect in lipogenesis (31). Since TPNH is required at many of the steps ✓ in the synthesis of the cholesterol molecule, a lack of this coenzyme could well account for the decrease in cholesterol synthesis seen in severe diabetes. On the other hand, in mild diabetes a *relative* deficiency

of *TPNH might* well be offset by the marked accumulation of cholesterol precursors caused by the block in lipogenesis, and an increase in cholesterol synthesis would therefore result. These relationships are shown in Figure 9-3 by the solid heavy arrows. This theory is supported by the evidence discussed in the next paragraph indicating that the excessive cholesterol synthesis of diabetes is closely related to the increase in ketone body production which is typical of this disease.

DIABETIC KETOSIS

Though the accumulation of the ketone bodies, acetoacetic acid, β-hydroxybutyric acid and acetone, is one of the most striking abnormalities of lipid metabolism to accompany the diabetic state, the biochemical

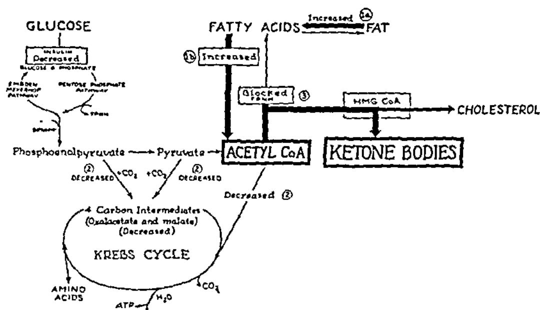


FIG. 9-5. Factors responsible for diabetic ketosis.

cause of this condition is still not completely understood. There is now no question that ketone bodies are derived primarily by synthesis from acetyl CoA in the liver, and ketosis must therefore result from the conversion of acetyl CoA molecules to ketone bodies by the liver at a faster rate than the ketone bodies can be oxidized by extrahepatic tissues. The rate of oxidation of ketone bodies is not significantly depressed by the diabetic state, and so this process can play no significant role in the development of ketosis. The synthesis of ketone bodies, on the other hand, is definitely increased in diabetes. This is probably the result of

an accumulation of acetyl-CoA which in turn must represent the net effect of increased production and inadequate removal of acetyl CoA by the liver cell.

The factors that influence the level of acetyl CoA and hence may cause ketosis are indicated in Figure 9-5. Acetyl CoA can be produced by the breakdown of any foodstuff; however, in diabetes, as discussed earlier, most must come from the catabolism of fat. The removal of acetyl CoA, on the other hand, can be accomplished by oxidation to carbon dioxide in the Krebs Cycle and by incorporation into fatty acids.

As discussed under "fatty acid oxidation" and illustrated in Figure 9-5, the rate of oxidation of fat is clearly increased in the diabetic over that in the normal animal. This is due both to an increased breakdown of fat to fatty acids as indicated by *1a* in Figure 9-5 and to an increased oxidation of the fatty acids themselves, *1b*.

The substitution of fat for glucose supplies the diabetic with adequate amounts of acetyl CoA and if the total carbon dioxide produced by a diabetic is assumed to be the same as that generated by a nondiabetic individual, the amount of acetyl CoA produced from this fat may even be somewhat in excess of normal.* An increased production of acetyl CoA may therefore be in part responsible for an increase of acetyl CoA concentrations in the diabetic cell; however, this point is by no means proved.

Both factors involved in the *disposal* of acetyl CoA, i.e., its oxidation to carbon dioxide and conversion to fatty acids, no doubt play important roles in the causation of diabetic ketosis.

In both the normal and the diabetic individual the Krebs cycle must serve as the primary source of carbons for various synthetic processes in the cell. In the normal animal, the four-carbon compounds so used are replaced by resynthesis of oxalacetate, a process that involves carbon dioxide-fixation either directly from phosphoenolpyruvate or via malate from pyruvate (reaction 2, Figure 9-5). In the diabetic, the absence of normal glycolysis may reduce the supply of phosphoenolpyruvate and pyruvate, as a result the Krebs cycle could become depleted of oxalacetate, and consequently the oxidation of acetyl CoA to carbon dioxide would be impaired.

The major arguments against the concept of a defect in the Krebs cycle of the diabetic are that total carbon dioxide production is probably normal in diabetes, and furthermore the conversion of acetate-C^{14}

* Only four of the six carbons of glucose are converted into acetyl CoA whereas all of the carbons of fat yield acetyl CoA. Therefore for an equal amount of carbon dioxide produced, glucose must contribute two and fat, three acetyl CoA molecules.

to $C^{14}O_2$ has been found to be normal in at least the diabetic rat (4). It should be pointed out, however, that owing to its mass-action effect a sufficiently increased concentration of acetyl CoA might allow normal carbon dioxide production despite an inherent defect in the Krebs cycle. In addition, evidence has been obtained indicating an impairment of conversion of acetate-C^{14} to $C^{14}O_2$ in diabetic dogs (14) and the direct determination of Krebs cycle intermediates has demonstrated a decreased level of these compounds in diabetes (12). Other workers have shown that the administration of some of the Krebs cycle intermediates to diabetics (17) or their isolated tissues (32) will reduce the degree of ketosis presumably by restoring the activity of the Krebs cycle. For reasons that are not clear this has not been the constant finding of all investigators. Finally, it is apparent that diabetes must at least result in a limited ability of the Krebs cycle to expand its activity during increased substrate demands since this pathway is obviously unable to remove the excess acetyl CoA that is present in diabetic ketosis. It must be concluded that at this stage in our knowledge a defect in the Krebs cycle provides the most reasonable explanation for the genesis of diabetic ketosis; incontrovertible evidence that such a lesion is consistently present in the diabetic animal has not, however, been published.

The necessity of the diabetic to utilize fat rather than glucose as his source of energy is also responsible for the other derangement in acetyl CoA disposal observed in this disease. As discussed earlier, the chief mechanism by which the normal animal is able to remove excess acetyl CoA molecules is by conversion to fatty acids. This pathway is no longer available to the diabetic and the resulting inability to store significant quantities of acetyl CoA as fatty acids is no doubt another factor in the development of the diabetic ketosis In a sense, the ketone bodies must under these circumstances serve both as the reservoir and as the transport form of acetyl CoA much as lactate serves an analogous purpose in the glycolytic pathway.*

The relationship between lipogenesis and ketone body formation is shown in Figures 9-3 and 9-5. A metabolic block on the pathway of lipogenesis at the site of TPNH action (see 3, Fig. 9-5), i.e., either at the conversion of crotonyl CoA to butyryl-CoA or in the reduction of acetoacetyl CoA would result ultimately in the accumulation of the acetoacetyl CoA formed by the coupling of acetyl CoA molecules. A third molecule of acetyl CoA could then condense with the acetoacetyl CoA to yield β-hydroxy-β-methylglutaryl CoA (HMG CoA). An excessive

* This analogy can be carried further in that β-hydroxybutyric acid, like lactate, can store and transport hydrogen ions derived from the DPNH generated during fat breakdown; DPN can thereby be regenerated for further fatty acid breakdown.

production of acetoacetic *acid* then results from the breakdown of HMG CoA to give the ketone body plus a molecule of acetyl CoA (21). As Lynen has now shown, the older view that acetoacetic acid is derived directly by deacylation of acetoacetyl CoA is probably not correct (21). Reduction of the acetoacetic acid by reduced diphosphopyridine nucleotide leads to the accumulation of the second ketone body, β-hydroxybutyric acid, and finally acetone is produced by spontaneous decarboxylation of the acetoacetic acid. The liver has a very limited ability to reactivate acetoacetic acid to acetoacetyl CoA (reaction 8, Fig. 9-1) and so, once formed, the ketone bodies are released into the blood to be either oxidized by extrahepatic tissues or excreted.

Figures 9-3 and 9-5 also serve to emphasize the fact that the chemical pathways of ketogenesis and of cholesterol synthesis are intimately interlinked. It is apparent from this relationship that conditions causing a blockage of lipogenesis produce an accumulation of β-hydroxy-β-methylglutaryl CoA, which in turn should lead both to the production of ketosis and, if small amounts of TPNH are present, to an increase in cholesterol synthesis as well. This expected parallel relationship between ketogenesis and cholesterogenesis is well borne out in the case of the diabetic animal.

The factors leading to diabetic ketosis can then be summarized as follows. The inability to utilize adequate amounts of glucose makes it necessary for the diabetic to rely on increased fat oxidation for energy. Fat can provide ample amounts of acetyl CoA but can supply neither pyruvate nor TPNH. As a result, the Krebs cycle is unable to maintain its integrity, and acetyl CoA can be oxidized to carbon dioxide only after the concentration of this compound is sufficiently high. The lack of TPNH prevents this excess acetyl CoA from being converted into fatty acids, as would normally occur under these circumstances, and the accumulated acetyl CoA is thereby shunted into HMG CoA and hence into the synthesis of ketone bodies.

SUMMARY

This chapter has attempted to review the present status of our knowledge of the diabetic defects in lipid metabolism. The viewpoint emphasized here is that both biochemically and etiologically these lesions are very closely interrelated. It has been pointed out that each of the major abnormalities in diabetic lipid metabolism, that is, the blockage in fatty acid synthesis, the increase in fatty acid oxidation, the development of ketosis, and the increase in cholesterol synthesis are probably all secondary to the reduction in glucose oxidation within the diabetic cell.

More specifically, many of the abnormalities of lipid synthesis would seem to be explainable on the basis of a decrease in the reduced triphosphopyridine nucleotide normally generated over the pentose phosphate pathway of glucose oxidation.

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Chapter 10

INSULIN AND PROTEIN METABOLISM

M. E. Krahl

INTRODUCTION

Insulin is required to permit synthesis of large molecules at optimum rates in mammals. It favors formation of storage and structural materials such as glycogen, fats, proteins, and hyaluronic acid. In diabetes, where the concentration of insulin available to the tissues is subnormal, these synthetic processes fail.

In man and animals the development of severe diabetes is associated with evidence of defective protein synthesis. The growth of young animals is slower than normal and the nitrogen balance tends to be negative. These stigmata of diabetes can usually be corrected by suitable doses of insulin, although the favorable effect of insulin upon protein synthesis is sometimes difficult to maintain when the diabetic patient is subject to stress.

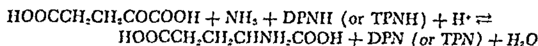
The classical findings regarding protein metabolism in diabetes are summarized in textbooks (2) and general reviews (12, 14). The discussion in this chapter will be concerned with present concepts of the physiological and biochemical mechanisms by which insulin influences protein synthesis.

PROTEIN FORMATION IN MAMMALIAN TISSUES

Amino Acid Supply

Eight amino acids must be supplied in the diet of man; they are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane, and valine. The others can presumably be synthesized in the mammal (in part by intestinal flora) by the following reactions (5):

REDUCTIVE AMINATION. α-Ketoglutaric acid from the Krebs cycle is converted to L-glutamic acid:



$$HOOCCH_2CH_2COCOOH + NH_3 + DPNH \text{ (or TPNH)} + H^+ \rightleftarrows HOOCCH_2CH_2CHNH_2COOH + DPN \text{ (or TPN)} + H_2O$$

TRANSAMINATION. Pyruvic acid, from the glycolytic cycle, can react with L-glutamic acid to give L-alanine



$$CH_3COCOOH + HOOCCH_2CH_2CHNH_2COOH \rightleftarrows CH_3CHNH_2COOH + HOOCCH_2CH_2COCOOH$$

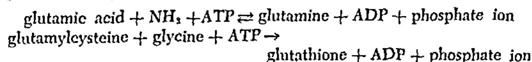
L-Aspartic acid is also synthesized from oxalacetic acid of the Krebs cycle in this manner. Other amino acids participate with α-ketoglutarate in transamination to some degree and may possibly be formed by this reaction.

SPECIALIZED REACTIONS. Glycine, histidine, serine, cystine, proline, hydroxyproline, and tyrosine are formed by individual reaction sequences (5).

Synthesis of Peptides and Protein (3)

SIMPLE PEPTIDES. New peptides can be formed by the reversal of proteolytic reactions (Bergmann and Fruton), for example, benzoyl-L-tyrosylglycine anilide is formed from benzoyl-L-tyrosine + glycine amide in the presence of chymotrypsin. This type of reaction is favored by removal of the peptide from solution, either as a precipitate or as part of a large molecule of low solubility.

Peptide synthesis can be coupled with liberation of inorganic phosphate from high energy phosphate compounds. Examples are (3):



$$\text{glutamic acid} + NH_3 + ATP \rightleftarrows \text{glutamine} + ADP + \text{phosphate ion}$$

$$\text{glutamylcysteine} + \text{glycine} + ATP \rightarrow \text{glutathione} + ADP + \text{phosphate ion}$$

The individual enzymes for each of these reactions have been found in liver.

PROTEINS. Protein synthesis in mammalian tissues is as yet poorly understood. Since the net increase in protein per unit time is too small to measure in isolated tissues where the synthetic conditions can be ex-

plicitly controlled, the synthesis of large molecular weight peptides has been studied by measuring the incorporation of labeled amino acids into acid-insoluble protein fractions. According to Borsook (3), the findings by this technique are: First, every tissue tested incorporates into its proteins, in vitro as well as in vivo, every common L-amino acid; second, some amino acids that are not normal protein constituents are not incorporated, e.g., α-amino adipic acid and α-amino isobutyric acid; third, the rate of incorporation varies from 0.1 to 10 micromoles per gram protein per hour, depending on the amino acid and tissue; fourth, the incorporation of a single amino acid is largely independent of the presence of the others in the incubation medium; fifth, *labeled amino acids are incorporated into peptide linkage*; sixth, in most cases inhibitors of respiration and phosphorylation inhibit amino acid and protein synthesis; there is evidence that in some cases cofactors are involved.

The mechanism of incorporation of labeled amino acids into protein fractions has been studied further, for example in microsomes from liver by Zamecnik and co-workers; under their conditions the required components are:

The labeled L-amino acid.

ATP, or a source of ATP, as energy donor.

A soluble enzyme system to activate the amino acid.

Guanosine diphosphate or triphosphate (GDP or GTP); the role of these is not known.

Microsomes; these incorporate the amino acid into a ribonucleoprotein. The microsomes lose this activity if the ribonucleic acid is destroyed by ribonuclease.

The degree to which these findings apply to other tissues or tissue fractions is not known. However, it is clear that protein synthesis can be regulated by a number of factors, each of which may be in turn dependent on processes regulated by insulin (10).

INSULIN EFFECTS ON PROTEIN METABOLISM IN WHOLE ANIMALS

The literature up to 1952–1953 has been reviewed by Lukens (12) and by Soskin and Levine (14). References to individuals who are mentioned here without individual citations will be found in these reviews.

Diabetic Men and Animals

In human patients, nitrogen loss is most pronounced in those having diabetes of the juvenile unstable type; those having the more stable type, especially if the disease appears late in life, often do not exhibit

excess urinary nitrogen loss. The question considered here is how insulin influences protein synthesis and breakdown. Early studies were carried out on pancreatectomized animals. More recently, alloxan-diabetic animals have been widely used; the latter have a variable amount of residual insulin depending on the severity of the diabetes. Since the *completely pancreatectomized animal lives no longer than a week or two*, it may be concluded that an alloxan-diabetic animal that lives more than a few days has some residual insulin; there is evidence that traces of insulin persist in the serum of rats rendered maximally diabetic with alloxan (11). Thus, although alloxan-diabetic animals are useful for studying effects of *relative* insulin deficiency (the situation that obtains in most human diabetics), they cannot be depended upon for studies where it is to be assumed that all insulin is excluded. Some of the principal findings for *severely* diabetic animals follow.

1. During fasting, glucose and nitrogen are excreted in the urine in amounts approaching the classical D/N ratio of 3.65, as would be expected if the urinary glucose were formed by catabolism of protein. However, this ratio is extremely variable and there is evidence that a limited fraction of the urinary glucose appearing under such conditions can arise from fat (1). To be converted to glucose, tissue protein is first broken down to amino acids; these are converted to the corresponding keto acids, either by oxidation or transamination; the glucogenic keto acids are further metabolized to the point where they can return to the glycolytic cycle as pyruvate, or to the Krebs cycle as oxalacetate or α-ketoglutarate, whence their constituent carbons can by reversal of the glycolytic cycle in liver (and possibly to some extent in kidney) be incorporated into glucose (see Chap. 8).

2. Administration of insulin to the fed diabetic human of the juvenile type or to the depancreatized animal restores nitrogen balance and permits growth of the young animal; this corrective effect can be achieved only with insulin, and not by feeding glucose, fructose, or oral hypoglycemic agents of the sulfonylurea type.

3. From studies on the disposal of N^{15}-glycine there is some evidence that protein synthesis is defective and protein breakdown excessive in diabetic animals (6); this conclusion is consistent with the findings on isolated tissues (see below).

Normal Animals

The effects of insulin on protein metabolism depend on the previous nutritional state of the animal. Administration of insulin does not cause demonstrable protein anabolism in the fasted animal (12). In the fed animal:

1. Insulin tends to produce a positive nitrogen balance, but long-continued administration does not promote growth in well-fed normal animals. It does cause some growth and nitrogen deposition in hypophysectomized rats; this effect is related to an increase in appetite and food intake (13).

2. Insulin reduces the accumulation of blood NPN in nephrectomized dogs (Mirsky) and of blood amino acids in eviscerated rats (Ingle).

Interrelationships of Insulin and Other Hormones with Respect to Protein Metabolism*

GROWTH HORMONE (GH). (1) GH produces nitrogen retention in fasting normal rats, differing in this respect from insulin, which does not. (2) GH does not produce nitrogen retention in diabetic dogs or cats, but can do so if a small dose of exogenous insulin is given concurrently (Lotspeich, Lukens). Thus, to produce its anabolic effect, GH has an absolute requirement for insulin. Insulin, on the other hand, can produce some nitrogen retention in the absence of GH but requires GH to produce optimum nitrogen retention and growth in the diabetic animal. (3) GH can induce metahypophyseal diabetes in certain animals (9).

Testosterone, like GH, also fails to exhibit its anabolic effect in completely depancreatized dogs (Sirek and Best).

ADRENAL CORTICAL HORMONES AND ACTH. (1) The negative nitrogen balance of diabetic animals tends toward normal when the animal is adrenalectomized (Long and Lukens) or hypophysectomized (Houssay). (2) Administration of 11-oxygenated adrenal steroids tends to increase nitrogen excretion in normal rats (Ingle), other animals, and normal man (Thorn, Conn); ACTH has similar effects. The negative nitrogen balance induced by large doses of adrenal steroids is not counteracted by insulin, even though the glycosuria accompanying the nitrogen loss may be abolished (Ingle). This anomalous separation of the effects of insulin upon glucose utilization and nitrogen balance may be due in part to the fact that adrenal steroids have strong catabolic effects upon some tissues, such as lymphoid tissues (4), in which insulin does not promote protein synthesis.

Stress causes a shift toward negative nitrogen balance, i.e., an increase in net protein breakdown, in diabetic human subjects and in animals This increase in nitrogen loss is dependent on the presence of permissive amounts of adrenal steroids, but there are other precipitating factors which are unidentified (7).

* See Chapters 16 and 17.

INSULIN EFFECTS ON AMINO ACID, PEPTIDE, AND PROTEIN METABOLISM IN ISOLATED TISSUES

This subject has been reviewed by Krahl (10).

Labeled Amino Acid Incorporation into Various Tissues

Insulin promotes the in vitro incorporation of amino acids (C^{14}-glycine or C^{14}-phenylalanine) into a protein fraction of rat muscle, liver, uterus, or seminal vesicle, but not spleen or intestine (Tidball and Krahl). The amino acid so incorporated has been found in peptide linkage. More complex tissue preparations such as perfused liver have also been studied (15).

Mechanism of Insulin Effect on Amino Acid Incorporation

MUSCLE FROM *Fed* NORMAL ANIMALS. Insulin promotes incorporation of various amino acids into excised rat diaphragm muscle, even in the absence of glucose in the incubation medium. Glucose alone is without effect upon amino acid incorporation under these conditions (Wool and Krahl). This effect of insulin on nitrogen anabolism is thus independent of that on glucose transport and utilization (Chap. 7) and represents *one facet of the general action of insulin to initiate intracellular molecular rearrangements that favor anabolic processes* (11).

MUSCLE FROM *Fasting* NORMAL ANIMALS. Insulin alone does not stimulate amino acid incorporation; glucose alone does; insulin and glucose together have a still greater effect.

LIVER SLICES. Incorporation of C^{14}-glycine into glutathione or various amino acids into liver protein is subnormal in liver slices from diabetic rats. This is apparently due chiefly to failure of protein synthesis rather than to increased protein breakdown. Insulin alone, added in vitro to liver slices from *mildly* diabetic rats, does not stimulate amino acid incorporation, glucose alone does, insulin and glucose together have a still greater effect. But when the diabetes is very severe or longstanding neither insulin nor glucose has any effect in vitro. However, the capacity to incorporate amino acids into liver protein is restored by injection of insulin into the liver donor several hours prior to the time of liver removal and test.

RÉSUMÉ. On the basis of these and related experiments discussed elsewhere (10, 11), there appear to be at least three insulin effects on amino acid incorporation into proteins of mammalian tissues. These concepts, although not in dispute, may be subject to modification as new evidence accumulates.

1. An effect on amino acid incorporation per se that is independent

of any effect on glucose transport; this is obtainable in muscle from fed animals which have an adequate energy supply in the form of glycogen. This effect may be concerned with amino acid transport from the extracellular to the intracellular phase, with release from inhibition of one or more enzymes concerned with protein synthesis (see Protein Formation, Transamination, above), or with as yet undiscovered mechanisms.

2. An effect that is dependent on the concurrent presence of glucose or other metabolizable sugar in the medium; this effect is at present ascribed to facilitation of glucose utilization, which in turn provides the necessary energy supply and favors accumulation of the necessary cofactors for peptide synthesis.

3. An effect to restore the capacity of diabetic liver to synthesize peptides at the normal rate; this effect requires more time than the first two, has so far been obtained only in intact animals, and appears to be one which is concerned with restoration of the machinery for protein synthesis (10, 11).

SUMMARY

1. Protein formation in mammalian tissues has been shown to depend on an adequate amino acid supply to the protein-synthesizing systems of tissues. The amino acids are provided as exogenous essential amino acids, or by syntheses involving either reductive amination and transamination or specialized individual processes.

2. The detailed reactions for protein synthesis in animal tissues are poorly understood. At present, the requirements include: amino acids, adenosine triphosphate or other energy donor, one or more enzymes to activate the amino acids, ribonucleoprotein, and possibly other cofactors.

3. Protein formation is subnormal in animals, particularly in certain animal tissues, when insulin is deficient; it is restored to normal by administration of insulin. To obtain optimal protein synthesis under the influence of insulin, the pituitary growth hormone is also necessary.

4. The action of insulin to promote protein formation is made up of a number of effects. These may include: facilitation of amino acid transport into cells, activation of enzymes for peptide synthesis, facilitation of glucose utilization to provide an adequate energy supply, and other undiscovered effects.

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Chapter 11

INSULIN ACTION: EFFECTS ON INDIVIDUAL TISSUES*

Albert E. Renold and Albert I. Winegrad

INTRODUCTION

The observed metabolic effects of any hormone are the result of its structurally determined ability to influence specific biochemical and biophysical processes in responsive tissues, and of the characteristic manner in which these tissues respond to the primary hormonal effect. The importance of tissue responsiveness as a determinant of the physiologic role of a hormone is well illustrated by the two hormones of the posterior pituitary: vasopressin (i.e., antidiuretic hormone) and oxytocin. These two hormones of known and remarkably similar structure have individually the capacity to influence the activity of both the kidney and the uterus. However, at physiologic concentrations of oxytocin the major response to this hormone is recognized as uterine contraction. The observed effect reflects the greater sensitivity of the myometrium to oxytocin, for at higher concentrations the effect on the renal tubular cells emerges and is reflected as antidiuresis. On the

* Supported in part by the Adler Foundation, Inc., Rye, New York; United States Public Health Service, Bethesda, Maryland, and the Nutrition Foundation, Inc., New York City.

other hand, physiologic concentrations of vasopressin produce antidiuresis well before uterine (or milk-ejecting) activity can be detected (40).

The administration of a hormone frequently produces a number of metabolic alterations of which one or at best a few are the direct result of hormone action, whereas the others are the *secondary* consequences of the *primary* effect or effects. A major purpose of metabolic-endocrine investigation is often that of establishing clearly the primary or secondary nature of metabolic effects observed in the presence of hormones. The delineation of the sequence of metabolic effects *in time*, both in the intact organism and in isolated tissues, has frequently been of value in helping to select likely candidates for primary sites of hormone action and to rule out unlikely ones.

From such considerations one may logically deduce that the total biologic importance of a hormone cannot be fully understood until information has become available concerning the presence or absence of effects of this hormone upon all tissues, individually; the nature, time of onset, and duration of these effects in each instance; and the hormonal concentration at which they occur. In progressing toward this goal, the definitive demonstration of *non-responsiveness* of a tissue to concentrations of the hormone that may be expected under either physiologic or pathologic conditions is as important as the definitive demonstration and characterization of responsiveness.

It is the purpose of this chapter to outline briefly the authors' present understanding of the physiologic characterization of *insulin* action at the level of individual tissues. Since these effects are obviously best studied in systems providing adequate isolation of otherwise intact tissues, major emphasis will be given to results obtained from in vitro techniques, utilizing surviving tissue preparations, cell suspensions, or perfused organs. Although species differences with regard to endocrine physiology and pathology are in part related to differences in hormonal structure, they probably derive much more frequently from *differences in the responsiveness* of one or several tissues. Information in this area is scant as yet, although it would seem likely that careful analysis of hormone-tissue interaction, in different tissues *and* in different species, might result in significant progress in our understanding of the over-all mechanisms of hormone action.

INSULIN RESPONSE OF INDIVIDUAL TISSUES

Some of the information available to the authors is summarized in Table 11-1 and will be elaborated upon in this section. It should be

TABLE 11-1. RESPONSIVENESS TO INSULIN OF INDIVIDUAL TISSUES*

Tissue	*Responsiveness to insulin*	*Major insulin effect*	*Effect, or lack of effect, demonstrated*			*Concentration of insulin required (microunits per ml.)*	*Time required for initiation of effect*
			in vivo	*in vitro*	*in man*		
Muscle							
Skeletal	Present	Increased glucose uptake, increased glycogenesis. Also: Increased uptake of amino acids	Yes	Yes	"Yes"	10–1000	Minutes
Heart	Present		Yes	Yes	Yes	60	Minutes
Adipose tissue	Present	Increased glucose uptake and oxidation, increased lipogenesis in the presence of glucose. Also: decreased release of fatty acids	Yes	Yes	"Yes"	<10	Minutes
Mammary gland (lactating)	Present (rat) Absent (sheep)	Increased lipogenesis in the presence of glucose		Yes		2000	Minutes
Leukocytes	Present	Increased glucose uptake		Yes	Yes	>10000	<1 hour
Lens	Present	Increased glucose penetration		Yes		>10000	<1 hour
Blood/aqueous	Present	Increased glucose transport	Yes				<1 hour
Tumors	Probably present in some	Increased glucose utilization		Yes		250	<1 hour
Brain	Absent		Yes	Yes	"Yes"		
Kidney	Probably absent		"Yes"		"Yes"		
Intestinal mucosa	Probably absent		"Yes"		"Yes"		
Erythrocytes	Absent		Yes	Yes	Yes		
Liver	Probably present but physiologic significance disputed	Increased glycogenesis (rabbit), increased lipogenesis and increased protein synthesis		Yes		>10000	See text

* This table represents the authors' interpretation of the available data. When the question as to demonstration of an effect is answered by "yes" rather than yes, the evidence on which the answer is based is largely indirect in nature.

clearly understood that this table represents the authors' present interpretation and digest of a large number of observations and reports and that, within the scope of this brief outline, reference can be made to only a few of the many outstanding contributions in this field. Furthermore, less importance has been given to information resulting from comparisons of the diabetic with the normal state than to information resulting from direct incubation of tissues with the hormone or from effects obtained shortly after the administration of insulin to the intact organism. When comparing tissues obtained from normal and diabetic animals it is particularly difficult to pinpoint lesions as being *directly* related to insulin deficiency, since diabetes mellitus is indeed a new metabolic state, which must comprise numerous secondary adjustments required by the primary metabolic defect (or possibly defects) resulting from insulin lack. For a more extensive bibliography the reader is referred to a recent, more fully documented review (33). Perhaps it should also be pointed out here that most tissue preparations still suffer from "impurity" and really comprise several tissue components, since all contain at least blood vessels and various connective tissue structures in addition to the major tissue fraction under investigation. Thus it has been pointed out by Levine that the major participation of ubiquitous connective tissue structures in most hormone effects (and particularly in the effects of insulin) has not as yet been adequately ruled out.

Skeletal Muscle

This tissue is the best-studied example of an insulin-responsive tissue. That insulin directly affects glucose entry and utilization in skeletal muscle was first established beyond reasonable doubt by Gemmill in the rat diaphragm, although earlier observations in "mixed" tissue preparations (perfused limbs or eviscerated organisms) led to similar conclusions. The responsiveness of skeletal muscle to insulin has now been studied in several species, including—as yet indirectly but very suggestively—man. In at least some laboratories insulin effects upon diaphragm tissue have been obtained at concentrations within the expected physiologic range for human plasma (50–1000 microunits per milliliter) and recent observations from Cori's laboratory have suggested that this may prove to be uniformly the case if special precautions are observed during excision of the tissue (22). It has also been established that insulin *rapidly* affects skeletal muscle metabolism and that a very brief period of contact with insulin-containing solutions will suffice to influence the metabolism of isolated muscle. The nature of the insulin effects observed will not be discussed here in detail (27, 35) (see Chaps.

7 and 14) and it may be adequate to state that most insulin effects described have been related more or less directly to increased glucose entry and/or its intracellular metabolism via existing pathways. However, some effects upon pyruvate oxidation (41), amino acid concentration (21), and amino acid incorporation into tissue protein (23), as well as effects upon permeability of the muscle cell membrane to large molecules such as the enzyme aldolase (47), have been described as occurring in the total absence of glucose.

Heart Muscle

The insulin-responsiveness of this tissue has been clearly established in, at least, rat, dog, and man. In man, myocardial metabolism can be adequately isolated by comparing arterial blood samples with simultaneously obtained samples from the coronary sinus, and, as a result, the direct responsiveness of this tissue to insulin is well documented (3). A particularly elegant tissue preparation that has been used to study myocardial metabolism is the isolated, contracting, and work-performing, perfused rat heart, developed by Bleehen and Fisher. In this preparation insulin effects can be shown at concentrations of 60 microunits per milliliter in the perfusing fluid and within 15 minutes of its addition to this fluid, as illustrated in Table 11-2.

TABLE 11-2. RESPONSIVENESS OF THE ISOLATED PERFUSED RAT HEART TO INSULIN*

Insulin concentration (*microunits per milliliter*)	*Glucose utilized* (*milligram per gram dry weight per hour*)
0	12 ± 1.2†
20	12 ± 2.0
60	21 ± 2.1
200	32 ± 3.3
600	35 ± 3.6
2,000	41 ± 2.6
20,000	49 (2 experiments only)

* From Bleehen and Fisher, *J. Physiol.* *123* 260, 1954.
† Mean ± standard error of mean.

In closing this discussion of the insulin-responsiveness of muscle, a rather paradoxic situation should be pointed out: although there can be no reasonable doubt about the ability of insulin to *increase* glucose utilization by skeletal and cardiac muscle, the functional integrity of both of these tissues appears to be well maintained even in states pre-

sumably characterized by prolonged and complete insulin deficiency, such as severe diabetic ketoacidosis (as long as severe alterations of extracellular electrolyte levels have not occurred). Furthermore, it would appear that cardiac muscle may perform useful work for several hours (without depletion of its glycogen stores) when perfused with solutions entirely free of glucose. Finally, cardiac glycogen is increased in the diabetic and in the fasting state.

Whether or not smooth muscle, and particularly vascular smooth muscle, also responds to changing concentrations of insulin in the surrounding fluid, has not yet been clearly established, although this information would appear to be particularly desirable.

Adipose Tissue

Although insulin effects upon adipose tissue metabolism, with regard to the deposition of glycogen and fat, has been accepted for many years (20, 44) the realization that this effect of insulin is a *direct* one upon adipose tissue—affecting glucose uptake, oxygen consumption, glycogen deposition, and fat synthesis—is relatively recent (14, 19, 23, 32). Indeed, it has now been established that the magnitude of the insulin effects upon nontraumatized, nonchilled preparations of adipose tissue equals or surpasses known insulin effects in any tissue (44) and can be reproducibly elicited at insulin concentrations as low as 10 microunits per milliliter or less (29). Furthermore, effects of insulin on this tissue occur within minutes of contact between the tissue and the hormone (Fig 11-1) and insulin effects have also been obtained with specimens of human adipose tissue obtained at operation. Thus adipose tissue may be considered a tissue whose insulin-responsiveness is as well established as that of muscle and which is likely to represent a site of insulin action of major physiological importance.

In contrast to glucose metabolism in muscle, glucose metabolism in adipose tissue is characterized by the very active participation of the phosphogluconate-oxidative pathway ("shunt"). This is consonant with the specialized nature of this tissue—for lipogenesis—and the importance of the phosphogluconate-oxidative pathway as provider of reduced triphosphopyridine nucleotide (reduced TPN) in the synthesis of fatty acids (Chap. 9). It is not surprising, therefore, that the interrelations between insulin and carbohydrate and fat metabolism have been documented with particular clarity in this tissue. As illustrated in Figure 11-2, insulin added in vitro exhibits its effect upon increased lipogenesis from acetate *only in the presence of glucose.* Glucose alone (without insulin), stimulates lipogenesis from acetate, and insulin stimulates lipogenesis from glucose both in tissue obtained from normal

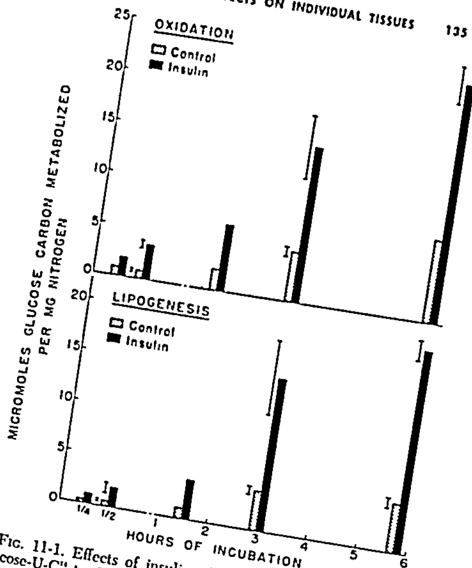


FIG. 11-1. Effects of insulin added in vitro on oxidation of glucose-U-C^{14} to $C^{14}O_2$ and on incorporation of glucose carbon into lipid by rat adipose tissue, after incubation periods varying from 15 minutes to 6 hours. All values are expressed as micromoles of glucose carbon per milligram tissue nitrogen. The incubations were carried out in Krebs-Henseleit bicarbonate buffer containing 20 millimoles per liter of glucose-U-C^{14}. Insulin concentration when present was 0.1 unit per milliliter.

and in tissue obtained from diabetic animals, despite the extremely low starting level in the latter instance. This series of observations in adipose tissue clearly suggests dependence of the insulin effect on lipogenesis upon the concurrent activation of glucose metabolism, an activation that results in an increased rate of glucose-6-phosphate oxidation (which has been demonstrated in this tissue) and, as a consequence, in an increased rate of TPN reduction. Thus, these results

would appear to add confirmatory evidence (in a metabolically somewhat simpler tissue) with regard to the dependence of lipogenesis upon the availability of reduced TPN, a dependence first postulated in a tissue metabolically much more complex, the liver.

It is of interest also that Gordon has demonstrated an inhibitory effect of insulin upon fatty acid *release* from adipose tissue in vitro, at least in

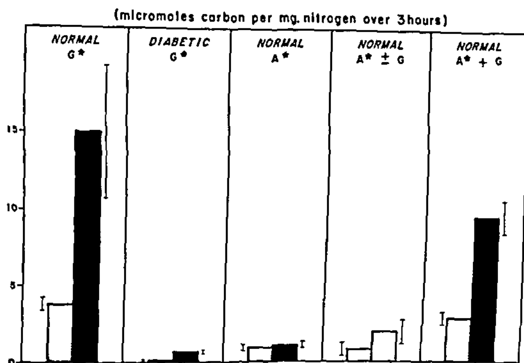


Fig. 11-2. Effects of insulin added in vitro on lipogenesis by rat adipose tissue. In all but the second pair of columns the tissues were obtained from normal rats; in the second pair of columns the tissues were obtained from rats with alloxan diabetes. Substrates: G* = 20 millimoles per liter glucose-U-C^{14}; A* = 60 millimoles per liter acetate-1-C^{14}; G = 10 millimoles per liter unlabeled glucose. The light bars refer to tissues incubated without insulin, the black bars to tissues incubated with insulin (0.1 unit per milliliter). The height of each bar indicates the micromoles of labeled substrate carbon incorporated into fatty acids per milligram nitrogen over 3 hours. Standard errors of the mean are indicated.

the presence of glucose. This last observation may be related to the dramatic response of plasma fatty acids (unesterified or nonesterified) to insulin administration, a response which is currently thought to result from decreased peripheral mobilization of lipid stores (12) concurrent with increased glucose utilization. Inhibition of fatty acid release from adipose tissue may well represent a major factor in the control of ketoacidosis by insulin administration.

Mammary Gland

An effect of insulin upon mammary gland tissue obtained from lactating rats is well established (1, 15). The presence of insulin in vitro results in increased fatty acid synthesis, increased CO_2 production, increased activity of the phosphogluconate-oxidative pathway, and a distinct elevation of the respiratory quotient. This effect is again dependent upon the presence of glucose in the medium. Although the effect of insulin upon this tissue has been particularly well studied, some question as to its physiological significance arises from the rather high concentrations of insulin required and from the apparent specificity of the effect for tissue obtained from lactating rats. Neither tissue obtained from nonlactating rats nor tissue obtained from lactating sheep demonstrates this responsiveness to insulin. However, one should keep in mind that the intermediary metabolism of ruminants and its response to insulin is generally peculiar.

Leukocytes

The study of white blood cell metabolism and of its regulation is of particular interest, since it concerns a tissue easily sampled in man and thus potentially open to routine investigation in health and disease. Decreased glucose utilization by leukocytes obtained from patients with severe diabetes mellitus has been described and a direct response of normal and diabetic cells to insulin present in the incubation medium has been elicited, although not in all instances. The required concentration of insulin is high, 0.1–0.5 units per milliliter (13). Undoubtedly, these studies are complicated by the difficulties encountered in the handling of white blood cells during the necessary separative procedures.

Lens

The decapsulated rabbit lens responds directly to the in vitro addition of insulin with an accelerated glucose uptake, whereas the acceleration was only one tenth as great with homogenized lens tissue. This strongly suggested to Ross that the effect of insulin in this tissue was principally on the cell membrane (Chap. 7). The *undecapsulated* rat lens does not respond to the addition of insulin in vitro. However, the intravenous injection of insulin two hours prior to the removal of the lens does stimulate glucose uptake by the undecapsulated lens. Further evidence that insulin does influence carbohydrate metabolism of the undecapsulated rat lens has come from the demonstration of a marked decrease in glucose uptake in the lens of alloxan-diabetic rats.

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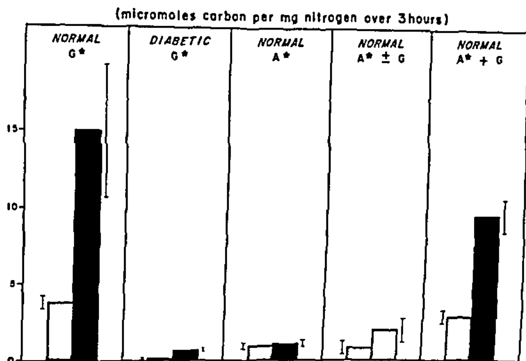


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Blood-Aqueous Barrier

The effect of insulin on the transfer of free glucose across the blood-aqueous barrier of the ciliary body of the rabbit eye is one of the major pieces of evidence supporting the so-called permeability hypothesis of the action of insulin (35), (Chaps. 7, 14). This complex epithelial structure exhibits a characteristic selective permeability and offers considerable resistance to the penetration of solutes into the aqueous humor. A direct effect of insulin on this tissue has been invoked to explain the fact that glucose is an exception to the generalization that the rate of solute penetration is proportional to the lipid solubility of the solute. Pretreatment of the intact animal with insulin raises the transfer constant for free glucose. Moreover, the rate of transfer of free glucose across the blood-aqueous barrier is sharply decreased in the alloxan-diabetic rabbit.

Tumors

Interest in the presence or absence of a direct insulin effect on specific tumor cells has been stimulated by the recognition that a very high rate of glycolysis (lactic acid production from glucose) under aerobic as well as anaerobic conditions is a fundamental property of tumor cells. Insulin does not affect the rate of penetration of glucose into the cells of the Ehrlich's ascites tumor, and appears to be without effect in this tissue (9).

When mice bearing melanoma S91 are exposed to high temperatures (35° to 40° C.) for 12 to 20 hours the in vitro anaerobic glycolysis of slices of excised tumor is markedly lowered, but the addition of insulin to such slices restores the rate of glycolysis to levels of tumors not exposed to high temperatures. This effect has been observed in a restricted group of tumors. Recently it has been reported that both S91 melanoma cells and mitochondrial preparations obtained from them respond to the in vitro addition of insulin (approximately 250 microunits per milliliter) with an increased rate of anaerobic glycolysis. It has been suggested that this represents an action of insulin on mitochondrial hexokinase, but the data do not exclude the possibility of an effect on mitochondrial permeability (5).

Brain

It is generally accepted that glucose utilization by the brain is not affected by insulin in vivo, and attempts to demonstrate an in vitro action of the hormone in this tissue have been unconvincing. It has been suggested that the intracellular free glucose concentration of the rat

brain may be appreciable, particularly when compared with that of the insulin-sensitive diaphragm, and is not altered by the in vivo administration of insulin (31). Although other hexoses such as fructose can support the respiration of brain preparations in vitro, only mannose (27a) relieves hypoglycemic cerebral manifestations in eviscerated, nephrectomized animals. It is now known that fructose does not pass the blood brain barrier, and that its entry into the brain is not facilitated by insulin (31). Although the acceptance of the nonresponsiveness of brain to insulin is widespread, it should be recorded that a few dissonant observations have been reported (46).

It is of course entirely possible that special areas of the brain are insulin-responsive, as has been suggested for the hypothalamic centers ✓ regulating food intake. Furthermore, the responsiveness to insulin of rat spinal cord (31a) and rabbit peripheral nerve (14a) has been recently reported.

Kidney and Intestinal Mucosa

The joint discussion of these two tissues may be justified by the small amount of information available on them, and by the need to consider either as both a glucose-utilizing and glucose-transporting tissue. With regard to metabolism other than glucose transport, it would appear that kidney slices obtained from diabetic rats differ from kidney slices obtained from normal rats (39) while a direct insulin effect on kidney slices has not been demonstrated. With regard to glucose transport, divergent statements are available in the literature, but the authors are inclined to accept the physiological observation that neither intestinal ✓ glucose absorption nor tubular glucose reabsorption is seriously impaired in the diabetic state, as evidence for the relative physiologic insignificance of whatever insulin-response these functions may exhibit. It would seem to us that *negative* information from diabetic animals is acceptable, since it rules out both primary and secondary insulin effects.

Erythrocytes

This tissue has been extensively investigated with regard to glucose transport and metabolism and is still being investigated as one of the best and most accessible models for this and similar transport systems. A reproducible insulin effect on erythrocytes has yet to be established, however.

Liver

Whether or not hepatic tissue is a directly insulin-responsive tissue is not as yet clearly established. Although a detailed discussion of this

much argued topic (6, 8, 10, 11, 18) appears hardly justified here, an outline may be helpful in restating the difficulties encountered in separating primary from secondary insulin effects and in establishing the insulin-responsive nature of a tissue.

It is generally accepted that liver tissue obtained from diabetic organisms is grossly abnormal with regard to the activity of dozens of metabolic reactions and pathways. These metabolic abnormalities include markedly decreased glucose phosphorylation, decreased glycogen synthesis and decreased lipogenesis, and increased glycogenolysis and gluconeogenesis. It is also generally accepted that normalization of all reactions that have been studied so far has been achieved by the administration of insulin in vivo. Although this would appear to be satisfactory prima facie evidence suggesting that insulin directly alters hepatic metabolism, certain objections have been raised. Firstly, this normalization of hepatic metabolism in diabetes by the administration of insulin in vivo requires *time* (34). Whereas the administration of insulin to diabetic rats for varying intervals prior to sacrifice *immediately* (within minutes) affects blood glucose levels and the metabolism of subsequently isolated skeletal muscle and adipose tissue, it does not demonstrably alter the metabolism of subsequently isolated liver until between 12 and 24 hours after the beginning of insulin administration, as illustrated in Figure 11-3. Furthermore, the secondary nature of the in vivo insulin effects has been established for some reactions, particularly for the normalization of fatty acid synthesis (2), and this could conceivably be true of all reactions affected by insulin in this tissue. Finally, one of the major mechanisms of insulin action presently postulated (i.e., facilitation of glucose entry into cells) could not be operative in this tissue, since the liver cell membrane does not offer the same barrier to glucose transfer into the cell as is the case, for instance, in skeletal muscle or for the blood-aqueous barrier. This has been recently rather definitely established (6) and is not unexpected in this tissue, which not only utilizes but also produces free glucose. The complexity of the situation, however, is further increased by consideration of the anatomic relationships. Insulin is secreted into the pancreatic and thus into the portal vein and cannot reach extrahepatic tissue without first perfusing the liver. The preservation of this relationship has suggested to some that insulin being secreted directly into the hepatic circulation *must* exert an important action directly upon hepatic tissue.

With this general background in mind, the following brief summary of the present state of our knowledge concerning the presence or absence of *direct* and *significant* insulin effects upon hepatic tissue may be helpful. Firstly, adequately controlled observations based upon direct

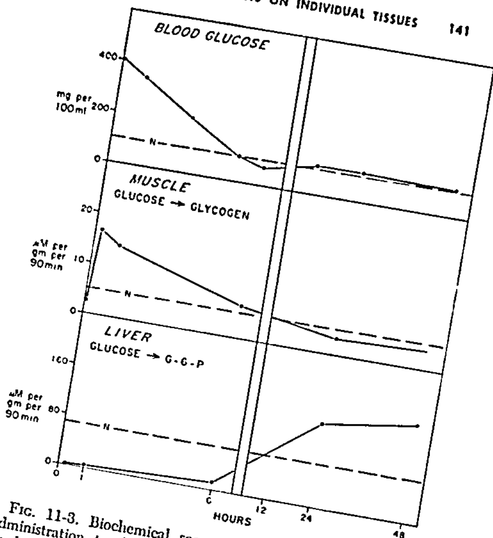


FIG. 11-3. Biochemical sequence of events after insulin administration to diabetic rats. *Top curve:* Blood glucose levels. *Middle curve:* Incorporation of substrate glucose into glycogen by the isolated diaphragm. *Bottom curve:* Glucose phosphorylation by isolated liver slices. In all instances the dotted line refers to the normal mean value and the initial point of the curve to control values obtained before insulin administration. For experimental conditions refer to Reference 34.

measurement across the liver in situ, although few, have as yet failed to reveal a direct effect of insulin upon hepatic tissue, particularly with regard to glucose uptake or output. Indeed, a most careful group of experiments recently carried out (36a) was interpreted by the authors as clearly suggesting the absence of any insulin effect upon hepatic tissue in their preparation. These experiments were carried out in resting, unanesthetized dogs, under experimental conditions that permitted the continuous simultaneous sampling of portal and hepatic venous blood

tainly not always negligible (as in the case of rabbit erythrocytes, for instance). Thus, from the point of view of tissue responsiveness, support is given to the concept of primary insulin action upon the transport of glucose across cell membranes. In considering the seeming discrepancy between the presence of direct effects of insulin on liver, whatever their physiologic significance, and the free permeability of the liver cell to glucose, it may be useful to recall that although a major portion of liver cell water appears to be freely accessible to glucose, small but functionally important segments of cell water (such as the mitochondrial space) might yet be surrounded by insulin-sensitive "membranes." The possible existence of further and different types of "compartments" within the cell has also been stressed by Stadie and Shaw (36).

Of similar interpretation is a second attempted generalization: that tissues which exhibit measurable levels of free intracellular glucose (erythrocytes, kidney, and perhaps brain) either are not insulin-responsive or only partly—at least differently—responsive (liver). This again would support an effect of insulin upon glucose penetration rather than upon glucose phosphorylation primarily, since cells with initially abundant intracellular free glucose would be ideal candidates for the latter effect. That the change in "permeability" to glucose may well be secondary to other intracellular metabolic changes has repeatedly been stressed by Cori and is of course rather generally accepted.

Thirdly, observations concerning the sequence of biochemic events in time as specifically related to tissues again give major prominence to skeletal muscle, heart muscle, and adipose tissue as major likely sites of primary insulin action. While the lag observed between insulin administration and insulin response in hepatic tissue suggests to the authors the possible physiologic insignificance of direct effects of insulin upon hepatic tissue, it is of interest to note that, in this tissue also, the first parameter showing alteration after insulin administration again relates to the earliest steps of glucose utilization, i.e., glucose phosphorylation. It should be pointed out here that the demonstration by Spiro of a lesser time lag between insulin withdrawal and hepatic response to insulin withdrawal than is true for the interval between insulin administration to diabetic animals and hepatic response to insulin does not alter the significance of the latter observation. In diabetic animals a discrepancy still exists between the rapid effect of insulin upon blood glucose level (usually taken to represent the major physiologic index of insulin action) and the slow response of hepatic carbohydrate metabolism. This discrepancy makes it difficult to explain the hypoglycemic activity of insulin in diabetic animals on the basis of a major hepatic action.

Fourthly, the observations that relate to the levels of hormone required significantly to alter the metabolic activity of any tissue may be of particular importance. Surely—and in analogy with the oxytocin/vasopressin example mentioned in the introduction—tissues that respond to insulin at low concentrations are more likely to represent a primary site of insulin action than are tissues whose response, however dramatic, can only be elicited at high concentrations of the hormone. On this basis the authors would single out adipose tissue as the tissue with the highest presently documented sensitivity to insulin. When only small amounts of insulin are available the most pressing physiologic need is for the prevention of fatal ketoacidosis, which probably is (or at least may be) prevented by decreasing mobilization of lipids from the fat depots, mostly from adipose tissue. The lesser tendency of the adult-onset type of diabetic to develop ketoacidosis may conceivably be related to his small remaining insulin reserve, inadequate for total blood glucose control, but adequate for adipose tissue effectiveness. It may be permissible also to point out here that inborn or induced variations in the responsiveness of a single tissue to a hormone might of itself produce significant metabolic abnormalities or disease states. Thus, uncomplicated hypersensitivity of adipose tissue to insulin might be expected to result in adiposity on the basis of increased trapping of glucose as depot fat, and decreased mobilization of depot fat once formed.

Fifthly, it may be of interest to point out that studies in isolated tissues have frequently been helpful in outlining more clearly the nature of certain metabolic interrelations and their connection to insulin action. Three examples will be mentioned. (a) The study by Stadie and his collaborators of the relationship existing between insulin which becomes "bound" to certain tissues and the occurrence of an insulin effect (38). The excellent correlation obtained in the case of rat diaphragm and rat adipose tissue left little doubt concerning the physiologic significance of the effects obtained in these tissues (Chap. 14) even though a similar correlation was not evident in all tissues which respond to the hormone. (b) Studies such as those of Mirsky and Williams (43), which have emphasized the tissue distribution of "insulinase" activity (Chap. 21) have underlined the importance of considering, in the hormone/target interaction, not only the activity of the hormone and the reactivity of the tissue, but also the duration of the interaction. It is of interest to note here that adipose tissue, in addition to exhibiting the greatest reactivity of tissues studied to date, also exhibits the smallest insulin-degrading activity. (c) The interrelation between metabolism and lipogenesis, which has been well demonstrated in hepatic tissue by, among others,

Langdon and Siperstein, and clearly related to the diabetic defect by Shaw and Gurin (Chaps. 9, 14), gains further metabolic characterization when considered in a tissue less complex than liver, more highly specialized with regard to the function studied, and unequivocally responsive to insulin in vitro, as previously discussed and illustrated (Fig. 11-2).

Finally, although much of the evidence discussed in tissues favors the primary effect of insulin upon an early step of glucose transport or utilization, and although it appears likely that the immediate hypoglycemic action of insulin is best explained on this basis, the authors would like to emphasize that this in no way excludes other sites and other mechanisms of insulin action. Some observations that cannot be explained on the basis of the known glucose effect alone have already been pointed out (17, 21, 23, 41). Further direct insulin effects could be arrived at either by extending the "permeability" hypothesis to include substances other than glucose, such as amino acids and even complex proteins, or by postulating entirely different and as yet unknown mechanisms of insulin action. The many existing discrepancies and paradoxes in the case of insulin effects upon hepatic tissue suggests that this tissue might be particularly suitable for unprejudiced reappraisal of the possible existence of alternate mechanisms of insulin effectiveness upon tissues. Such a reappraisal may already have been initiated (25, 27b).

SUMMARY

This chapter has stressed the importance of the target organ or tissue in the physiologic expression of hormone action, more particularly of insulin action. Some of the existing evidence concerning insulin-responsive and insulin nonresponsive tissues has been reviewed and summarized in Table 11-1. Particular importance has been given to the insulin-responsiveness of striated muscle on the one hand and adipose tissue on the other. Although more evidence has accumulated with regard to the former tissue, the authors have demonstrated some partiality to the possible paramount physiologic importance of the effects of insulin upon the latter. The general consideration of the information obtained in isolated tissues was thought to favor the concept of *a major* effect of insulin upon glucose transport into cells, while not excluding the presence of other effects of the hormone—be it transport of other substances or entirely different metabolic effects. The paradox existing with regard to hepatic tissue (as yet equivocal physiologic importance of direct insulin effects versus major metabolic derangements in diabetes and "strategic" anatomic location with regard to insulin secretion) was

felt to suggest the possible presence of a different and as yet inadequately characterized insulin action with particular importance in liver.

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Chapter 12

PLASMA LIPIDS

Vincent P. Dole

Most diabetics are slightly hyperlipemic, even when they appear to be in good clinical control. One would be inclined to ignore these minor changes since they cause no obvious symptoms, but an impressive amount of indirect evidence suggests that some disturbance of lipid metabolism might contribute to the development of arteriosclerosis—a complication that kills the majority of diabetics. If this is the case, these relatively slight changes in plasma lipids reflect serious disturbances of metabolism in the tissues. Statistical evidence supporting the hypothesis has been provided by a number of studies, all of which show a higher incidence of coronary artery disease in patients with elevation of blood lipids.

Before the availability of insulin, most diabetics died from an acute failure of lipid metabolism—ketosis. The problem has been only partially solved by modern treatment. Insulin protects against sudden death from acidosis, but it fails to prevent the recurrence of minor ketotic episodes that magnify the chronic disturbances of lipid metabolism. Patients with a history of irregular control usually show a persistent and rather marked degree of hyperlipemia; they also tend to develop arteriosclerosis early and in malignant form.

Clearly, diabetes is a general disturbance of metabolism, with the changes in lipids sometimes inconspicuous and in other cases dominant —for instance, in patients with so-called idiopathic hyperlipemia and mild diabetes. With advance of biochemic knowledge, no doubt we will be able to interpret these changes in functional terms, and replace the present, largely empirical effort to correlate chemical entities of unknown function with diseases of unknown cause.

CHEMICAL CLASSIFICATION OF PLASMA LIPIDS

Plasma lipids can be classified into five broad chemical categories: phospholipids, triglycerides (often called neutral fat), cholesterol (either free or esterified with fatty acids), nonesterified fatty acids (NEFA), and a miscellaneous collection of other lipid-soluble materials in smaller quantity (fat soluble vitamins, carotenoids, cerebrosides, sterols). A complete analysis of this mixture would be exceedingly difficult and quite impractical for most clinical studies. In general the entities selected for clinical study have been those most easily determined in the laboratory: cholesterol, lipid phosphorus, or a combination of two or more categories, such as triglycerides plus cholesterol (similar solubilities), total fatty acids (easily titrated), or simply total lipids (determined by weight, turbidity, or centrifugal separation). Such a grouping of categories obviously diminishes the empirical value of the results and obscures their functional significance.

Despite limitations of the data, the triglyceride fraction stands out as having special importance in the pathogenesis of diabetes. This fraction often is increased to a significant extent in patients who show normal concentrations of cholesterol and phospholipids; with greater severity of the disease triglycerides accumulate to a striking degree (Table 12-1). The turbidity or creaminess of diabetic plasma, often reported, is due to elevation of triglyceride concentration.

Unfortunately, triglycerides are somewhat difficult to measure. They give no distinctive reactions that permit their determination in a mixture, and they are not easily separated from other lipids. As a result the literature abounds in cholesterol data, but presents relatively few reliable studies of triglyceride. Better methods of analysis just now becoming available promise to remove the difficulty. It may be anticipated that the importance of triglyceride in diabetes and arteriosclerosis will become better defined in the near future.

Nonesterified fatty acids (NEFA) are closely associated with triglycerides in metabolism, and appear to be of considerable interest in relation to diabetes Although they comprise but a small part (about 5

per cent) of the total fatty acids in blood plasma, the NEFA exchange at an exceptionally fast rate (half-time about 2 minutes) and probably account for a significant portion of fatty acid transport. Their intimate association with carbohydrate metabolism can be shown by simple experiments: when glucose is fed to a normal subject the NEFA fall sharply, correlating with the rise of glucose in the blood; injection of insulin causes a parallel fall of NEFA and of glucose, while injection of epinephrine causes a rise of both.

TABLE 12-1. PLASMA LIPIDS IN DIABETES*

	Normal controls	*Uncomplicated diabetes*	*Diabetes and retinopathy*	*Diabetes and Kimmelstiel-Wilson syndrome*
Number of cases	11	38	12	16
Cholesterol				
Total	245 ± 54	236 ± 49	256 ± 41	*318 ± 64*
Estrified	.	174 ± 37	187 ± 22	*221 ± 59*
Phospholipids	214 ± 38	259 ± 47	290 ± 33	*326 ± 61*
Total lipids	629 ± 136	*804 ± 182*	*958 ± 160*	*1195 ± 268*
Neutral fats	231	*310*	*411*	*550*

* Adapted from Adlersberg, David, *et al. Diabetes* 5.117, 1956

All values in units of milligrams per 100 milliliters The data in italics are regarded as significantly increased above normal SD shown.

Apparently the stream of NEFA originates in adipose tissue and flows to other tissues utilizing fatty acids for energy or synthesis, notably the liver. Control of the stream appears to be located in adipose tissue, since injection of insulin causes a reduced outflow of NEFA from depots into blood but no change in the rate of removal of labeled fatty acid already in circulation.

As would be expected, the NEFA fraction reflects the disturbance of carbohydrate metabolism in diabetes. Apparently well-controlled diabetics tend to have a somewhat elevated concentration of NEFA in samples of plasma taken before injection of insulin in the morning. If insulin is withheld from a severe diabetic for only a few hours, the concentration of NEFA rises sharply, paralleling the rise of blood glucose. In patients entering the emergency ward with severe acidosis or coma, the concentration of NEFA is likely to be increased to 2 or 3 times the normal value. Treatment of these patients with adequate doses of insulin causes a prompt drop of NEFA to normal, paralleling the fall in blood glucose, and anticipating by some hours the slower disappearance of ketone acids from blood and urine.

The association between rise of NEFA and subsequent appearance of ketosis suggests that NEFA might be precursors of the ketone acids. It is well known that in ketosis the utilization of ketone acids by peripheral tissues is normal, or nearly so, and that the liver is the only important source of ketone acids discharged into the blood. The problem, therefore, is to determine what factors in diabetic ketosis so greatly accelerate ketogenesis in the liver.

First, the abnormal *direction* of fatty acid metabolism in the diabetic liver appears to be explained by a defect in lipogenesis. Four-carbon acids tend to accumulate because synthesis of fatty acids beyond the four-carbon stage is impaired; a deacylase enzyme splits coenzyme A from the accumulated intermediates, trapping them as free ketone acids. The second variable, namely the *quantity* of ketone acids produced by such a blocked system, presumably is determined by the rate at which substrate is delivered to some pool behind the metabolic block. Studies of the NEFA fraction suggest that clinical ketosis may be precipitated by a failure in regulation of the rate at which fatty acids are released from adipose tissue. With deficiency of insulin, and possibly under the positive stimulus of other hormones, adipose tissue discharges fatty acids at a greatly increased rate; they appear in the blood as NEFA and are delivered to the liver in excess quantity, loading the organ beyond its limited capacity. If this interpretation is correct, a progressive rise of NEFA concentration in a diabetic should alert the physician to danger of impending ketosis. Conversely, in treatment of established ketosis the return of NEFA concentration to a normal level would provide assurance that the primary fault had been corrected by administration of insulin.

These, and a variety of other studies, emphasize the importance of adipose tissue in diabetes. Long represented as an inert reservoir of excess calories, adipose tissue is now becoming recognized as an organ with intense metabolism, responsive to hormonal and nervous control. The continuous turnover of fatty acids in adipose tissue—with an animal in caloric balance—caused much surprise when discovered some twenty years ago. Subsequent results have been even more surprising. Adipose tissue, per unit weight of protoplasm, exceeds liver in its rate of lipogenesis. It converts a major part of dietary carbohydrate to fatty acid before oxidation, and it may transform the dietary fatty acids before releasing them to the bloodstream. It responds to insulin with greater sensitivity than any other tissue. Indeed, the results suggest that synthesis and conversion of fatty acids are the primary functions of adipose tissue, and that its role as a storage organ is merely incidental.

The well-known relation between obesity and diabetes needs to be re-

examined. If adipose tissue is metabolically important and closely regulated, it seems unlikely that a gross accumulation of triglyceride within its cells would have no more reason than an accident of dietary excess. One finds on reducing obese subjects, diabetic or not, that the total metabolic rate usually falls to subnormal levels, suggesting that the accumulation might have been compensatory in some way, as yet undefined. It can be surmised, but not proved, that adipose tissue when loaded with triglyceride is in a functional state different from that when depleted. If so, the undernutrition treatment of diabetes, which proved lifesaving before the insulin era, may have worked by changing the functional state of adipose tissue, making it more responsive to limited amounts of endogenous insulin. Conversely, the appearance of manifest diabetes after gain of weight may be due to a change in the metabolic system responding to insulin rather than a decrease in the production of hormone.

The metabolic activity of adipose tissue in vitro is now being studied in a number of laboratories. With suitable systems one sees active lipogenesis in vitro, and a controlled discharge of fatty acids into the medium, influenced by insulin, epinephrine, and ACTH. As these mechanisms become better defined, the role of adipose tissue in diabetes certainly will become clarified, and quite possibly some of the changes of triglyceride and NEFA concentration in plasma can be used to measure its performance.

LIPOPROTEINS

Practically all the lipids in circulation are carried as components of lipoproteins—delicate complexes of phospholipid, cholesterol, cholesterol ester, and triglyceride associated with specific proteins. These highly organized structures are destroyed by the usual methods of chemical analysis, but they can be separated (apparently in native form) by the less violent processes of electrophoresis, ultracentrifugation, or Cohn fractionation.

With each method the lipoproteins divide into two groups. On electrophoresis one finds a faster component, the α-lipoproteins, moving just behind albumin, and a slower group, the β-lipoproteins. The same two groups separate in the ultracentrifuge, distributing themselves over a continuous spectrum of densities. The β-lipoproteins, being rich in triglyceride and relatively poor in protein, are of low density and as a result tend to float when the density of the medium exceeds 1.063 (the conventional density for measurement of flotation rates, designated by the symbol, S_f).

Broadly speaking, all these structures must be concerned with transport. Much of the lipid in plasma at any moment is on its way to somewhere—not just in the sense of moving with the bloodstream, but in the specific and directed sense of material delivered into circulation by some tissue and destined to be removed at a different place by another tissue. This much is clear but we do not know enough for more detailed clinical interpretations. It would be good to know what components of the lipoproteins are being carried and what parts serve as carriers by conferring solubility or chemical specificity. The rate of turnover is important, since the functional contribution of any element to transport is measured by its flux and not by its concentration. We would like to know what chemical relations exist between different lipoproteins, whether the real functional unit is a group of lipoproteins with easy interconversion, or whether the component molecules of any given complex stay together or exchange freely with the environment. The answers to these questions would carry us some distance toward rational interpretations of measurements made in the clinic.

In study of function it is convenient to distinguish primary transport of lipids—material absorbed from gastrointestinal tract and being delivered to various tissues—from secondary or retransport of material from the original depots to other tissues. After a meal containing fat, the concentration of triglyceride in blood plasma, and to a lesser extent the concentration of phospholipid, are increased for several hours. Evidently this represents primary transport. The plasma becomes turbid, and on microscopic examination is found to contain numerous chylomicrons, which in the ultracentrifuge rise rapidly to the surface because of their low density. On closer study of flotation rates one finds that the chylomicrons form a continuous series of structures of increasing density, merging into the β-lipoprotein group, and presumably representing different degrees of loading with triglyceride. The diabetic patient may or may not show a moderate increase in duration and amplitude of this absorptive process.

Apparently the more important defect in diabetes involves some kind of secondary transport. Chylomicrons, which are detectable in plasma of normal fasting subjects, are significantly increased in diabetics, even those in good clinical control. In ketosis the plasma is loaded with chylomicrons, often exceeding the quantity found after a heavy fat meal, despite the fact that the anorectic patient may have eaten nothing for many hours. Just where these structures come from and what they signify is still obscure. Quite possibly some light will be thrown on this phenomenon by future studies of the "clearing" process, in which

heparin and other sulfated polysaccharides facilitate the removal of chylomicrons from circulating blood.

Presumably the group of β-lipoproteins is concerned mainly with secondary transport, since its concentration is not much affected by alimentary lipemia. These lipoproteins appear to have similar compositions, and may prove to be a functional unit. The protein component of the various lipoproteins in the group cannot be distinguished either chemically or immunologically. The relative proportions of cholesterol, cholesterol esters, phospholipids, and protein are roughly the same over the range of different densities. The major variation in chemical composition of the β-lipoproteins arises from variation in content of triglyceride, which suggests that this group of lipoproteins might serve as a carrier system for triglycerides. Further tests of this hypothesis will be of great clinical interest; an increase of the triglyceride-rich, β-lipoprotein is the most consistent abnormality of plasma lipids in diabetes (Table 12-2).

TABLE 12-2. QUALITATIVE DESCRIPTION OF LOW DENSITY LIPOPROTEINS IN DIABETES AND IN CORONARY INSUFFICIENCY

Designation	*Density range*	*Triglyceride content (%)*	*Diabetes*			*Coronary insufficiency*
			Good control	*Irregular control*	*Ketosis*	
Chylomicrons S_f 20–400	<1.006	80	+	+	++	+
S_f 12–20	1 006–1 019	50	N	+	+	N
S_f 0–12	1.019–1 063	10	N	±	±	N

N = concentration within normal limits in most cases, + = moderate increase in many cases; ++ = marked increase; ± = variable changes.

On the other hand, the high-density α-lipoproteins are almost a complete mystery and are not systematically affected in diabetes (so far as is known at present). Much richer in protein and lower in triglyceride and cholesterol than β-lipoprotein, and possessing chemically and immunologically distinct proteins, these structures are unmistakably different from members of the β group. However, the two groups are not wholly independent of each other, since labeled phospholipids migrate freely from one group to the other. A further possible association is suggested by the finding that the protein of chylomicrons (exceedingly small in amount) may be similar to the protein of α-lipoproteins.

SUMMARY

The most consistent change in plasma lipids of treated diabetics is a moderately increased concentration of triglycerides, which are carried principally by the β-lipoproteins. With more severe forms of the disease, increases of cholesterol and phospholipid appear, but in practically all cases, including diabetic ketosis, the excess of triglyceride predominates. Nonesterified fatty acids (NEFA) appear to be of interest, particularly because they may be involved in the production of ketosis. It seems quite probable that the increased amounts of triglycerides and NEFA in plasma reflect abnormalities in the metabolism of adipose tissue.

The reader interested in further detail will find chemical data and ample bibliographies in the first two references given below. The third reference summarizes recent studies of NEFA. The fourth is particularly valuable for its careful analysis of clinical studies, and in addition provides access to the literature on the chemistry of lipoproteins. The fifth describes recent studies exhibiting hormonal control of adipose tissue.

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Chapter 13

MUCOPOLYSACCHARIDE CHANGES IN DIABETES

Albert Dorfman

The metabolic homeostasis of complex organisms is dependent on the intracellular environment. This environment is conditioned not only by the circulation but by the connective tissue matrix in which cells are imbedded. It is now apparent that this connective tissue compartment is not solely an ultrafiltrate of plasma, but contains components that impart to the ground substance specific physical-chemical properties. The constituents of the ground substance determine the chemical and physical-chemical characteristics of the milieu surrounding metabolizing cells. All nutrients and waste products must pass through the ground substance while in transit between circulation and cell.

Recent studies have resulted in a rapid extension of knowledge concerning the composition of the ground substance. It is now apparent that this complex solution contains materials derived from the circulation, products of the metabolism of parenchymal cells, as well as substances peculiar to connective tissue. This discussion is concerned with the latter compounds.

An important constituent of the connective tissues is the fibers. Available data indicate the existence of two principal types of fibers: collagenous and elastic. The presence of soluble precursors of collagenous fibers has now been well established. Soluble precursors of elastin are

likely, although such compounds have not yet been identified. It is beyond the scope of this chapter to discuss the effects of insulin on protein metabolism but it should be emphasized that both collagen and elastin are protein in nature. It is to be expected that any agent or condition that affects protein metabolism might influence the metabolism of these substances.

The acid mucopolysaccharides of connective tissue are a group of high molecular weight linear polyelectrolytes.Their basic structure is indicated by Figure 13-1, which illustrates the disaccharide repeating unit of chondroitinsulfuric acid-A of cartilage. In general an acetylated amino sugar alternates with a uronic acid. Certain of the acid mucopolysaccharides also possess sulfate groups. An exception to this type of structure is keratosulfate, which contains a galactose moiety in place of a

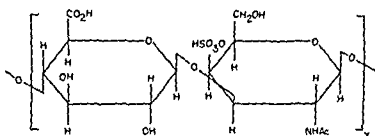


Fig. 13-1. The disaccharide repeating unit of chondroitinsulfuric acid-A of cartilage.

uronic acid. Table 13-1 lists the known acid mucopolysaccharides and their constituents. It is important to emphasize the diversity of these compounds. It seems likely that with increasing knowledge regarding the chemistry of the specific mucopolysaccharides, new avenues will present themselves for study of physiologic functions and pathologic disturbances. It is already apparent that this group of substances varies in biologic activity, e.g., chondroitinsulfuric acid-A is completely devoid of activity in the blood coagulation system, while chondroitinsulfuric acid-B, differing only with respect to the configuration of C-5 of the uronic acid, is a potent antithrombic substance. Already it has been possible to show that, following hypophysectomy, growth hormone exerts a differential effect on the metabolism of hyaluronic acid and the sulfated mucopolysaccharides of skin.

Important in the consideration of the physiology of acid mucopolysaccharides is the fact that at least some of these compounds exist in tissues as firmly bound protein complexes. Such complexes have been variously called mucopolysaccharide-protein complexes or mucopro-

teins. The nature of mucopolysaccharide-protein complex has been most thoroughly investigated in cartilage. Shatton and Schubert first showed that chondroitinsulfuric acid-A is firmly bound to a noncollagenous protein by linkages other than electrostatic. More recently, Mathews has demonstrated that *chondroitinsulfuric acid-A exists in units of molecular* weight 50,000, which are bound to a protein core to make macromolecules with minimal molecular weights of 4,000,000. The studies of Muir suggest that the mucopolysaccharide chains are bound through serine residues of the protein.

TABLE 13-1. MUCOPOLYSACCHARIDES OF CONNECTIVE TISSUES

	Amino sugar	*Uronic acid*	*Sulfate*
Hyaluronic acid	N-acetylglucosamine	Glucuronic acid	−
Chondroitinsulfuric acid-A	N-acetylgalactosamine	Glucuronic acid	+
Chondroitinsulfuric acid-B (β-heparin)	N-acetylgalactosamine	Iduronic acid	+
Chondroitinsulfuric acid-C	N-acetylgalactosamine	Glucuronic acid	+
Chondroitin	N-acetylgalactosamine	Glucuronic acid	−
Keratosulfate	N-acetylglucosamine	(Galactose)	+
Heparin	Glucosamine (N-sulfated)	Glucuronic acid (?)	+
Heparin monosulfuric acid	Glucosamine	(?)	+

Investigations on the connective tissue changes in diabetes mellitus and arteriosclerosis have been confused by a lack of clarity in terminology. A number of investigators have assumed that the glycoproteins ("mucoproteins") of blood are products of the depolymerization of acid mucopolysaccharides of connective tissue ground substance. Early classifications applied the term mucoprotein to proteins containing in excess of 4 per cent of glucosamine.

More recently, the term glycoprotein has been suggested for this purpose while the term mucoprotein has been reserved for complexes of mucopolysaccharides and proteins. Methods that have usually been applied to measure blood glycoproteins (mucoprotein) do not reflect the presence of mucopolysaccharides in blood. Attempts to determine the presence of mucopolysaccharides in blood have demonstrated that extremely small amounts of these substances are normally present in blood. Bassiouni reported that an extract of 420 ml. of normal human plasma contained traces of two acid mucopolysaccharides that could be detected by paper electrophoresis. Similar findings were reported by

Bollet *et al.*, who reported an average level of 277 μg. of mucopolysaccharides in normal adult serum, measured as uronic acid. Schiller was able to isolate only 1.5 mg. of chondroitinsulfuric acid per liter of human plasma. The only evidence of larger amounts of mucopolysaccharides in blood is the report of Deutsch of the presence of hyaluronic acid in the sera of two patients, one with a reticulum cell sarcoma and the other with a neuroblastoma. Levels of blood mucopolysaccharide in diabetes mellitus so far have not been reported.

The assumption that blood glycoproteins result from the depolymerization of mucopolysaccharides of connective tissue seems most unlikely on the basis of knowledge of the chemistry of these two groups of substances. Available evidence indicates that blood mucoproteins contain glucosamine, galactose, and mannose tightly bound to protein. Unlike the acid mucopolysaccharides, long-chain carbohydrate fragments have not been obtained from the blood mucoproteins (glycoproteins). One of the blood mucoproteins has now been well characterized and has been named orosmucoid; it contains large amounts of scialic acid. The transformation of a long-chain mucopolysaccharide composed of alternating units of N-acetylgalactosamine (or glucosamine) and glucuronic acid to a glycoprotein composed of scialic acid, N-acetylglucosamine, galactose, and mannose requires profound chemical transformation. The fact that glycoprotein levels rise in conditions characterized by connective tissue changes in no way establishes a causal relationship between these two phenomena.

Although not necessarily related to connective tissue, mention should be made of a number of studies concerned with variations of blood mucoproteins and hexosamine in diabetes mellitus. West *et al.* showed that the concentration of glucosamine is abnormal in a variety of pathologic conditions. Since that time an extensive literature has indicated that mucoprotein levels are elevated in a variety of conditions. In 1949 Jacobs claimed that the level of bound glucosamine in blood increased in diabetics and correlated with blood sugar. He found in one patient that the level of glucosamine fell upon administration of insulin. The methods used by this investigator probably did not measure the total serum hexosamine-containing proteins. Berkman, Rifkin, and Ross, with more adequate methods, observed abnormal levels of serum glycoproteins only in diabetics with the Kimmelstiel-Wilson syndrome. A rise in the urinary excretion of hexosamine-containing compounds in diabetics was observed by Cottentot and Tanret. These authors found the hexosamine excretion was not related to blood glucose levels or the extent of glycosuria, but was more strikingly elevated in patients with complications.

More recent studies have confirmed the finding of the elevation of serum mucoproteins in diabetics, but have indicated that the extent of change is minimal in the absence of complications of diabetes. The significance of altered blood mucoproteins remains poorly understood. Recent literature is replete with studies demonstrating nonspecific changes in blood proteins in a variety of diseases, but little or no information is available regarding the mechanism of these changes.

The possible role of insulin in the biosynthesis of the carbohydrate portion of glycoprotein is unknown. The role of the liver in glucosamine metabolism is not entirely clear. There seems little question that certain of the enzymes and intermediates involved in glucosamine metabolism are present in liver. It is likely that these are concerned with fabrication of the carbohydrate portion of the glycoproteins. The studies of Wick and coworkers indicate that in both rat diaphragm and liver, glucosamine behaves as do glucose, mannose, and galactose in response to insulin. The intracellular transfer of glucosamine results in the inhibition of glucose transfer. It was suggested that glucosamine competes with glucose at the site of insulin action. Although studies of hexosamine metabolism in relationship to insulin are of considerable importance, they do not necessarily cast light on mucopolysaccharide changes in diabetes mellitus.

The decreased resistance to infection, disturbed wound healing, and rapidity of vascular degeneration in diabetes mellitus have led to considerable speculation regarding alterations in connective tissue ground substance in this disease. Unfortunately, no clear picture is yet available.

Older concepts that vascular degeneration results from alterations of the connective tissue have been largely eclipsed in recent years by the intense interest in the relationship between serum lipids and arteriosclerosis. The possible role of the ground substance has recently been emphasized by Faber, Taylor, Wartman, Aldersberg and co-workers, and others. For the most part, available histochemic methods have not been adequate to delineate the nature of changes in tissues.

Tanret and Cottentot have described a histochemic anomaly in the skin of diabetic patients, which is characterized by increased metachromasia These authors claim that this change is coincident with a decrease in sulfhydryl groups in the connective tissues.

Sendrail and Blum attempted to study the effect of insulin on connective tissue by utilization of the spreading reaction. They found a slight inhibition of the hyaluronidase-induced spreading phenomenon following the injection of insulin into normal individuals. The differences observed were probably not significant.

Recent investigations concerning the metabolism of acid mucopoly-

saccharides have clarified considerably the pathway of biosynthesis of these substances. Earlier studies on the biosynthesis of hyaluronic acid by Group A streptococci established the fact that both the hexosamine and uronic acid portions of this molecule derive from glucose without scission of the carbon chain of glucose.

More recent metabolic studies suggest that the hyaluronic acid molecule may be formed by the following series of reactions:

(1) glucose-1-PO_4 + UTP $\rightleftharpoons$ UDPG + UDP
(2) UDPG + 2 DPN $\rightleftharpoons$ UDPGA + 2 DPNH + 2 H^+
(3) fructose-6-PO_4 + glutamine $\rightleftharpoons$ glucosamine-6-PO_4 + glutamic acid
(4) glucosamine-6-PO_4 + acetyl CoA $\rightleftharpoons$ N-acetylglucosamine-6-PO_4
(5) N-acetylglucosamine-6-PO_4 $\rightleftharpoons$ N-acetylglucosamine-1-PO_4
(6) N-acetylglucosamine-1-PO_4 + UTP $\rightleftharpoons$ UDPAG + UTP
(7) UDPAG + UDPGA $\rightarrow$ hyaluronic acid

It is apparent from this series of reactions that synthesis of hyaluronic acid depends upon the availability of glucose as a phosphorylated intermediate. Irrespective of the mechanism of action of insulin, there appears to be little question of the role of insulin in making glucose available for intracellular metabolic reactions. It therefore seems reasonable that diabetes mellitus might be characterized by disturbance of mucopolysaccharides. An attempt to evaluate this possibility has been made by Schiller and Dorfman. In previous investigations, methods were devised for the estimation of the rate of metabolism of acid mucopolysaccharides in the skin of rats and rabbits. It was possible to show that hyaluronic acid is metabolized at a more rapid rate than are the sulfated polysaccharides of skin. The rate of metabolism was determined by the use of acetate-1-C^{14}, glucose-U-C^{14}, and $S^{35}O_4$. Each of these isotopic precursors was found to effect labeling of specific portions of the mucopolysaccharide molecules. The rate of metabolism measured by each of the precursors was similar, indicating that turnover of the entire molecule was being measured.

In order to study the influence of the diabetic state and insulin on mucopolysaccharide metabolism, alloxan-diabetic rats were employed. Compared to normal animals and half-starved controls there was a marked decrease in both the rate of uptake and of disappearance of isotopes. Similar results were observed whether glucose-U-C^{14}, acetate-1-C^{14}, or $S^{35}O_4$ were utilized as isotopic precursor. When insulin was administered to alloxan-diabetic rats the rate of mucopolysaccharide metabolism was restored toward normal.

These data strongly suggest that insulin is required for the maintenance of normal rates of metabolism of acid mucopolysaccharides The

TABLE 13-2. MUCOPOLYSACCHARIDES IN THE SKIN OF NORMAL, FASTED, AND DIABETIC RATS

Type of animal (20 rats/group)	*Aver. body wt. (Gm.)*	*Hyaluronic acid*			*Chondroitinsulfuric acid*		
		Concentration mg./100 Gm dry skin	*Radioactivity* C.P.M. at zero time*	*Turnover rate† mg./day/100 Gm. dry skin*	*Concentration mg./100 Gm. dry skin*	*Radioactivity* C.P.M. at zero time*	*Turnover rate† mg./day/100 Gm. dry skin*
Normal	355	59 6	6009	7.7	38.1	2032	2.4
Fasted	212	51 0	5811	9.3	55.3	1753	3.7
Diabetic	210	30.2	1968	4.6	25 6	769	2 3

* Refers to the C^{14} in the group of 10 rats sacrificed 24 hours after injection of radioactive acetate. All counts have been corrected for differences in body weight and are calculated on a per kilogram basis. All samples were counted as $BaCO_3$ in an internal gas-flow counter and were corrected to "infinite thickness."

† The turnover rate was calculated from the turnover time as the average quantity of each mucopolysaccharide synthesized and degraded/day/100 Gm. dry skin; where t_r = [polysaccharide]/t_t and $t_t = 1.44t_{1/2}$.

marked interference with biosynthesis suggested the possibility that an absolute decrease in the amount of mucopolysaccharides might be observed in diabetic animals. Since methods for the direct determination of mucopolysaccharides in tissues are not satisfactory, an isotope dilution method was devised for this purpose. Table 13-2 illustrates the results of such experiments. It is apparent that there is a striking decrease in concentration of acid mucopolysaccharides in the skin of alloxan-diabetic rats. The decrease in hyaluronic acid is relatively greater than that of chondroitinsulfuric acid. This observation finds ready explanation in the relatively more rapid turnover of the hyaluronic acid. The measurements of pool sizes permitted estimation of turnover rates, which are more appropriate indicators of synthetic rates than are half-life times. The calculated values shown in Table 13-2 indicate the severity of the defect of synthesis of acid mucopolysaccharides in the diabetic animal.

Recently, Juhlin has attempted to study the mucopolysaccharides in alloxan-diabetic rats by a quite different technique. This investigator has estimated the reconstitution of a hyaluronidase-sensitive barrier in skin. This represents that material which resists the spread of hemoglobin but is destroyed by the injection of testicular hyaluronidase. This was found to be greater than normal in diabetic animals. On the basis of these experiments the results of Schiller and Dorfman were questioned. No attempt was made, however, to correlate the extent of the hyaluronidase-spreading reaction with the concentration of mucopolysaccharides in skin. It is difficult to evaluate these results in comparison with quantitative metabolic experiments.

CONCLUSIONS

Numerous studies have indicated that the ground substance probably plays an important role in homeostasis in addition to its obvious anatomic functions. There seems little question that the acid mucopolysaccharides play a critical part in wound healing and resistance to diffusion of materials in connective tissues. Recent studies on the clearing reaction indicate a possible additional mechanism by which sulfated mucopolysaccharides may be involved in the metabolism of lipids. Evidence of a diversity of acid mucopolysaccharides with specific localization and biologic activities make possible highly selective functions as well as pathologic disturbances. Limitations of present knowledge precludes a detailed description of the physiologic implications of impaired mucopolysaccharide metabolism in diabetes. It is obvious that this biosynthesis is not limited to this group of substances. Nevertheless,

it seems reasonable that interference with normal rates of mucopolysaccharide metabolism may be of importance in the pathogenesis of certain changes observed in diabetes mellitus. In addition to obvious relationships to wound healing and infection, of more general interest is the possibility of a role in the pathogenesis of vascular degeneration.

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Chapter 14

SUMMATION OF INSULIN EFFECTS

William C. Stadie

INTRODUCTION

The problem of the action of insulin has been studied by innumerable research workers since the isolation of the hormone. Indeed, the search for a definition of insulin action anteceded the discovery of insulin in 1922. The extensive studies in the preinsulin days of the various aspects of diabetes by the chemist, the metabolist, and the clinician were studies of insulin lack. Such studies still continue to be pursued in many hitherto unexplored areas by modern techniques and continue to be informative.

The question: "What does insulin do in the mammalian organism?" receives different answers, dependent upon the interest, the purpose, the objectives, and the techniques of the worker who asks the question. The clinician asks: "What reduces blood sugar levels in the diabetic? How is the threatening ketosis diminished? Why is atherosclerosis more prevalent in the diabetic than in the normal patient? Why does a deficiency of insulin action favor the development of nephropathy, retinopathy, and neuropathy?" The chemist strives for a closer definition of the relation of the hormone to enzymatic systems. He may ask: "Is the Embden-Meyerhof glycolytic system catalyzing the initial steps in the metabolism

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have shown that the metabolic activity of iodinated insulin is the same as that of its native form. (2) By formation of sulfate esters using $H_2S^{35}O_4$, which combines with the alcoholic group of threonine. S^{35}-insulin has the advantage of having a half-life of 80 days compared to that of 8 days in the case of I^{131}-insulin. (3) The third method is to incorporate C^{14} into the insulin molecule. This is accomplished by using cultures of pancreatic tissue in a medium containing some amino acid labeled with C^{14}, e.g., C^{14}-alanine or glycine. The newly formed insulin containing the isotopic amino acids is then isolated from the tissue culture. The insulin prepared by this method contains low amounts of radioactivity and has been used in studies on the structural characteristics of insulin.

BOUND INSULIN (16)

A few *a priori* considerations make it likely that insulin, in order to be metabolically active, is bound to tissues. The old idea that enzymes, substrates, and hormones exist within the cell in homogeneous solution is no longer advocated. The current concept postulates that enzymatic systems are incorporated into cell surfaces or subcellular elements. The older concept makes it necessary to assume that insulin, enzyme, and substrate molecule must simultaneously collide for the hormone to influence the reaction. But trimolecular collisions are rare occurrences. It is more likely that the hormone and the enzyme form a hormone-enzyme complex which catalyzes the reaction of a substrate molecule, which comes within molecular distance of the complex. Whatever the nature of this complex is, it involves the binding of the hormone to the tissues.

In the case of the rat diaphragm, hormonally active bound insulin is demonstrated as follows (17): a hemidiaphragm is dipped into a solution containing insulin at concentrations varying from 0.001 to 0.1 units per milliliter. As little as 10 seconds' exposure to the insulin solution is sufficient to bind enough of the hormone to be metabolically active. The diaphragm may then be washed through many changes of wash medium without removing significant amounts of the bound insulin. The bound insulin is demonstrated by transferring the hemidiaphragm to a medium containing glucose. A hemidiaphragm that has not been treated with insulin in a preliminary period is used as control. The insulin-treated diaphragm will take up more glucose from the medium than its control, thus demonstrating the presence of metabolically active bound insulin. By using radioactive insulin, the hormone bound to the tissues may be quantitated. It can be shown, for example, that the increased glycogen synthesis from glucose by insulin-treated diaphragms is proportional to

INSULIN ACTION IN RELATION TO THE STRUCTURE OF THE HORMONE

A number of protein hormones influence the metabolism of mammalian tissue. It may seem extraordinary that these large molecules should have any effect upon enzymatic complexes which are themselves protein. However, it is known that proteins, even those of large molecular size, react chemically with both small and large molecules. The possibility that hormones become part of an enzymatic system to increase or decrease its activity is thus plausible.

The determination of the order in which the constituent amino acids of insulin are joined together in peptide linkage has been successfully achieved by Sanger (Chap. 2). The two chains, A and B, by themselves have no demonstrable action upon metabolism. Two striking aspects of the insulin molecule are the disulfide bridges that link chains A and B together and the intrachain disulfide bridge on chain A. The assumption that these disulfide linkages might form a complex with some enzyme system to alter its reactivity is an attractive one. However, at present nothing is known about the relation of the structure to the metabolic activity of insulin, except that minor variations in the make-up of the two chains in insulins obtained from different species do not change the physiological activity of the hormone.

ISOTOPIC INSULIN

In a number of problems the availability for experimental purposes of isotopic insulin is advantageous. For example: (1) the localization of insulin following equilibration of tissues in vitro or after intravenous injection in the intact animal. Such localization gives information on the metabolic activity of the hormone. (2) Isotopic insulin can be quantitated accurately after recovery from tissue. Insulin has been prepared with sufficiently high radioactivity so that as little as 0.01 μg. may be determined. Experiments relating metabolic activity to these minute amounts of insulin are thus made possible (3) The study of the metabolism of insulin itself can be done by following its concentration in tissues and urine after injection of the isotopic preparation into animals.

There are three ways in which insulin can be converted into radioactive forms. (1) by iodination with I^{131}. This is accomplished by incubating the insulin solution under suitable conditions with I^{131} followed by dialysis to remove the excess of free iodine The iodine combines with the benzene ring of the tyrosine. In practice, only about 1 out of 1,000 of the tyrosine residues in the insulin molecules are iodinated. Studies

bound insulin is in accord with our knowledge of its action obtained in other ways. For example, response to bound insulin is diminished in the alloxanized animal. Bound insulin activity is also diminished by the prior injection of cortisone and growth hormone into the rat before the diaphragm is tested. These hormones exert their counterinsulin action upon the insulin bound to the diaphragm. Hemidiaphragms from hypophysectomized rats show that amounts of bound insulin which in normal rats are without effect upon glucose uptake have almost maximal effects. This is in accord with the well-known insulin sensitivity of the hypophysectomized animal.

THE NATURE OF THE ENZYMATIC REACTIONS INFLUENCED BY INSULIN

Recent reports in the literature indicate that the action of insulin is primarily upon synthetic reactions requiring energy input, that is, endergonic reactions. This topic is discussed in relation to the influence of insulin upon protein synthesis (Chap. 10) and in the discussion of insulin action upon fatty acid synthesis (Chap. 9). At the present time it is not clear as to whether the effects of insulin upon the transport of glucose into the interior of certain cells requires the input of energy. But this possibility cannot be dismissed. Energy-rich phosphate compounds, such as ATP, comprise the ultimate source of chemical energy in intracellular metabolism. The demonstration by Vester and Stadie, that there is diminished oxidative phosphorylation in mitochondria prepared from the livers of depancreatized cats lends considerable support to the concept under discussion. The evidence is more striking in that the defect in oxidative phosphorylation can be eliminated by restoring the diabetic animals to a normal state by adequate insulin treatment.

Many attempts have been made to explain all the metabolic defects observed in diabetes on the basis of one single hypothesis. It is becoming increasingly clear that these attempts are difficult if not impossible. It is highly probable that diabetes is associated with a variety of metabolic defects that result in slowing or cessation of key enzymatic reactions. However, if all these reactions are endergonic, it is quite possible that they may have one basic causation, namely, a deficient availability of energy-rich compounds capable of supplying the necessary energy for the reaction.

INSULIN ACTION UPON GLUCOSE METABOLISM

There can be no doubt that insulin is concerned with an early stage of the metabolism of glucose, but whether or not this is its sole action

the amount of insulin bound (Fig. 14-1). Using the same technique, it is possible to demonstrate that other tissues, e.g., adipose and mammary tissues, bind metabolically active insulin. The distribution of injected radioactive insulin shows that tissue with high rates of metabolism, e.g., liver and muscle, bind large amounts of insulin. The binding capacities

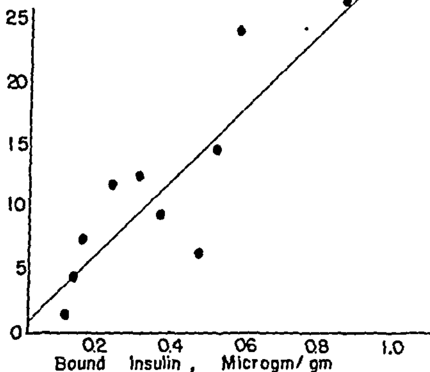


Fig. 14-1. Effect of insulin bound by rat diaphragm on the synthesis of glycogen by rat diaphragm in vitro (17).

of the structural elements of the liver, e.g., mitochondria, nuclear fraction, and microsomes, are different, indicating a specificity of binding capacity. Leukocytes bind more insulin than any other tissue. Recently it has been found that conversion of glucose to lactic acid by leukocytes from severely diabetic subjects is significantly less than normal values. This together with the observation that brain, which is insulin-nonresponsive, does not bind insulin, lends support to the hypothesis that the binding of insulin is necessary for its hormonal activity.

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bound insulin is in accord with our knowledge of its action obtained in other ways. For example, response to bound insulin is diminished in the alloxanized animal. Bound insulin activity is also diminished by the prior injection of cortisone and growth hormone into the rat before the diaphragm is tested. These hormones exert their counterinsulin action upon the insulin bound to the diaphragm. Hemidiaphragms from hypophysectomized rats show that amounts of bound insulin which in normal rats are without effect upon glucose uptake have almost maximal effects. This is in accord with the well-known insulin sensitivity of the hypophysectomized animal.

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and the mechanism by which it is achieved are still matters for discussion and experimentation. The first reaction of glucose in mammalian tissue and one necessary for its further metabolism is the formation of glucose-6-phosphate through the action of glucokinase. This reaction has long been regarded as a site of hormonal regulation, including acceleration by insulin. There are two ways in which this acceleration may be accomplished: (1) by increasing the rate of entry of glucose into the cell through the cell membrane. This aspect of insulin action has been discussed (Chap. 7). (2) The second method of insulin action is to increase the rate of formation of glucose-6-phosphate by direct action upon the enzyme hexokinase. This acceleration by insulin may be a release of the inhibitory action of some other hormone, such as pituitary or adrenal factors. But it is also possible that the influence of insulin upon the hexokinase reaction may be an indirect one in that it accelerates the rate of formation of ATP by its influence upon oxidative reactions by which this high-energy compound is formed.

Recently evidence has accumulated that insulin may have a direct action upon the enzymatic systems concerned with the initial two oxidative steps in the hexose monophosphate shunt. These two reactions lead to the formation of TPNH without which the synthesis of fatty acids is seriously impaired. The significance of this aspect of glucose metabolism is fully discussed in relation to fat metabolism in Chapter 9.

RELATION OF INSULIN TO PHOSPHATE METABOLISM

There is a close relationship between the metabolism of phosphate and carbohydrate not only in mammalian tissue but in yeast, microorganisms, and other living organisms It is, therefore, no surprise that departures from the normal relationship between phosphate and carbohydrate metabolism are found in the mammalian diabetic animal It has been further demonstrated that insulin significantly affects the phosphate metabolism in both normal and diabetic animals. The earlier literature on this subject has been reviewed by Stadie (15). Metabolic schemas outlining the intermediary steps of glucose metabolism (see Fig. 8-1) show that it is possible to assume that a defect in oxidative phosphorylation which generates high-energy phosphate in the form of ATP might explain most if not all of the observed effects in the metabolism occurring in the diabetic individual. For example, an impairment of ATP formation would slow the initial phosphorylation of glucose and thus decrease its rate of incorporation into the metabolic pathway leading either to glycogen synthesis or to production of pyruvate. Furthermore, synthesis of fatty acids and proteins has been shown to be dependent

upon the availability of ATP. It is even possible to postulate that ATP is concerned in a "transfer mechanism" so that transport of glucose into the muscle cell would be impaired if a decrease in ATP formation occurred as part of the diabetic syndrome. Tracer studies in the intact animal with isotopic phosphate (P^{32}) has given much information on the interrelation of phosphate and carbohydrate metabolism. Chief among workers in this field are Kaplan and Greenberg (7, 8). They injected radioactive phosphate into the intact animal and determined its uptake by liver and muscle and also the distribution of the label in various inorganic phosphate fractions. Studies were done on fasted, postabsorptive, and diabetic animals. In general insulin always tended to increase the turnover rate of P^{32} in the various organic phosphate fractions of liver and muscle. Data from their experiments show, for example, that in the fasted state insulin resulted in a significant increase of the labeled P in ATP. This increase was suppressed if malonate was injected simultaneously with the insulin. The animal on a high-fat diet showed the same general results but to a more striking degree. The fact that the malonate inhibited the action of insulin was suggestive of the possibility that oxidative phosphorylations occurring in the Krebs cycle were influenced by the hormone. Sacks (9) using isotopic phosphate reported on its incorporation into the intermediary phosphate compounds of muscle. In summary, his data indicate that the rate of uptake of inorganic phosphate from serum is depressed in the diabetic state and increased by the administration of insulin. Intermediary esters such as glucose-6-phosphate and glucose-1-phosphate are increased. The report of Haugaard, Marsh, and Stadie leads to the same general conclusion. They used diaphragms from normal rats equilibrated in glucose with or without insulin. They measured ATP, phosphate esters, and glycogen before and after equilibration in vitro. They calculated that in the presence of insulin there is an extra formation of high-energy phosphate measured as ATP. Following insulin, Goranson, Hamilton, and Haist found an increased incorporation of injected radioactive phosphate into ATP and phosphocreatine in the normal fasted rat. They concluded that insulin accelerates oxidative phosphorylation. Charalampous and Hegsted using the intact normal and alloxan-diabetic rat also concluded that insulin is concerned with oxidative phosphorylation. They studied a reaction known to be dependent upon ATP, namely the acetylation of para-aminobenzoic acid. They determined in the alloxanized rat the percentage of injected acetylated para-aminobenzoic acid excreted in the urine. In the diabetic there was a significant decrease in acetylization, which returned to the normal value following injection of insulin. They concluded "that a primary deficiency in ATP formation is the cause of

the metabolic defect." Phosphorylation of thiamine in the experimental rat requires ATP. On the basis of this observation, Foa, Weinstein, Smith, and Greenberg studied the total thiamine and the percentage of phosphorylated thiamine in the normal and alloxanized diabetic rat. They found a highly significant decrease in phosphorylation from the normal value of approximately 82 per cent to 34 per cent. Siliprandi and Siliprandi also found that the phosphorylated thiamine content of

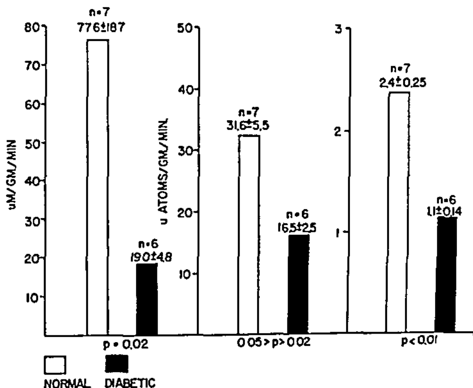


Fig. 14-2. Oxidative phosphorylation by hepatic mitochondria from normal and diabetic cats (19). Left-hand column, ~P formation measured as ATP formation; middle column, oxygen uptake; right hand column, P:O ratio.

liver in alloxanized diabetic rats was increased by insulin. Goranson and Erulkar also studied the formation of high-energy phosphate as phosphocreatine in homogenates of heart from normal and alloxanized rats. In all instances they observed in the alloxan-diabetic rat a highly significant decrease in phosphorylation of creatine using either succinate or malate as the oxidizable substrate. Insulin increased the rate of phosphorylation in these homogenates both in normal and in alloxanized diabetic animals.

It is generally believed that the reactions resulting in synthesis of

high energy phosphate bonds occur exclusively in mitochondria. Vester and Stadie studied the possibility that this activity in hepatic mitochondria is impaired in the diabetic animal. For this purpose, they used the depancreatized cat and isolated the mitochondria from liver homogenates using the customary methods of differential centrifugation. The washed mitochondria were suspended in a suitable medium containing pyruvate as a source of energy and glucohexokinase to combine

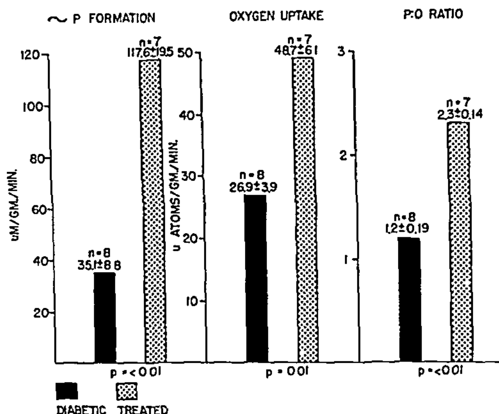


FIG 14-3. Restoration to normal of the oxidative phosphorylation by mitochondria prepared from livers of insulin-treated depancreatized cats (19).

with the ATP formed. After incubation for 60 minutes at 38°, the ATP generated, the oxygen uptake, and the P:O ratio were determined (Fig. 14-2) The rate of formation of ATP by the mitochondria from the liver of the diabetic cat (left-hand column) was reduced compared to the value in the normal animal. The P:O ratio, which measures the efficacy of the oxidative phosphorylation by the mitochondria, was also significantly reduced. The authors further showed that after treatment of the cat with insulin (Fig. 14-3) the depressed values of oxidative phosphorylation were essentially restored to the normal values. The ex-

periments were performed as follows: Two or three days pancreatectomy a biopsy on liver was done and from this ma mitochondria were prepared and assayed for their oxidative phorylative ability. The animal was then vigorously treated with in for five or six days and was then sacrificed. Studies on the mitochondria were repeated. The results are shown in Figure 14-4 indicate a return of oxidative phosphorylation toward normal follo the insulin treatment.

These data, together with other data in the literature, indicate the reactions concerned with oxidative phosphorylation are imp and that this impairment is a factor in the departure from normal of carbohydrate, and protein metabolism in the diabetic animal.

DUAL ENZYMATIC SYSTEMS WITH IDENTICAL ACTION COEXISTING IN THE SAME TISSUE

Until recently it was tacitly assumed that only one system of enzy and cofactors catalyzing a reaction or series of reactions existed single type of tissue. For example, only one Embden-Meye glycolytic system in muscle dissimilates glucose through the bra point glucose-6-phosphate either to glycogen or lactic acid. Likew only one enzymatic system in liver or other tissue oxidizes higher f acids to acetyl Co-A or, operating in the opposite direction, synthes them from available precursors of acetyl Co-A. Recent experim have been reported indicating that under certain circumstances identical systems may coexist in a given tissue, differing either in nature of their cofactors or their location upon cytostructural eleme of the cell. In consequence, the two systems differ in significant deg in their catalytic function of response to hormones. An example of phenomenon is illustrated by observations on the glucose metaboli of the normal isolated rat diaphragm equilibrated in vitro in eithe bicarbonate-phosphate-saline medium or a phosphate-saline medi containing no bicarbonate (10). The two systems are identical to classical Embden-Meyerhof system but differ in two important respec (a) the first system is intracellular; (b) the second system appears be located upon the surface of the cell. In addition, in a bicarbon medium the first system contains all the enzymes of the Embd Meyerhof scheme. It, therefore, catalyzes the formation of glycogen a of lactic acid through the branch point glucose-6-phosphate. In t phosphate medium, however, the first system is incomplete: it conta no active phosphoglucokinase. The intracellular system is responsive insulin. This is shown (using isotopic glucose in the medium) by

increased rate of turnover of glucose-6-phosphate, glucose-1-phosphate, fructose-1,6-diphosphate, as well as an increased synthesis of the end products, glycogen and lactic acid. This insulin-responsiveness is attributed to the action of the hormone, which increases the transport of glucose from the medium to the interior of the cell. In the phosphate medium, as was noted, the intracellular system is incomplete in that it lacks an active phosphoglucokinase. In consequence, insulin does not increase the production of lactic acid, since none is produced by this intracellular system. The second or extracellular system converts isotopic glucose to lactic acid through glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate. It does not synthesize glycogen from glucose-6-phosphate nor does it convert glucose-6-phosphate to glucose-1-phosphate. Further, since no transport of the medium glucose is needed for enzymatic formation of lactic acid from glucose, this surface system is nonresponsive to insulin. Whether these two identical systems, each responding differently to a hormone, function in vivo is unknown.

Another dual enzymatic system of great significance in relation to diabetes which coexists in the same tissue is the one concerned with the oxidation of higher fatty acids to acetyl Co-A or, operating in the reverse direction, the synthesis of fatty acids from acetyl Co-A. So far as we know, the apoenzymes that catalyze the intermediary steps in either direction between these respective end substances are identical. However, it is highly presumptive from evidence discussed in Chapter 9 that the cofactors in these two systems are different. Those of the oxidizing system are DPN and flavin adenine dinucleotide. Further evidence could be interpreted to indicate that the enzymatic system concerned with the oxidation of long-chain fatty acids to ketone bodies exists only in the mitochondria. Presumably on account of the lack of the cofactors which are present in the synthesizing system, they are unable to perform the reverse reaction of synthesizing higher fatty acids from acetyl Co-A. The system of enzymes that synthesizes fatty acids is presumably extramitochondrial. This intracellular but extramitochondrial system depends for its ability to synthesize fatty acids upon a supply of reduced TPN This cofactor, reduced triphosphopyridine nucleotide, is supplied if there is concomitant metabolism of glucose through the hexose monophosphate shunt.

Under these circumstances, there is a balance of fatty acid synthesis in the extramitochondrial cell substance and the oxidation of fatty acid within the mitochondria. Homeostasis with respect to fatty acid metabolism is established and there is no ketosis. However, if glucose metabolism is absent because of lack of insulin action on glucose, the

necessary reduced $TPNH_2$ is not supplied and fatty acid synthesis ceases. Oxidation of fatty acids to acetyl Co-A terminating in the formation of ketone bodies goes on unbalanced by synthesis; ketosis results. Apparently the two enzymatic systems, which have presumably identical apoenzymes but have different cofactors as well as locations within the cell, cannot interchange their functions.

The possibility of similar dual enzymatic systems coexisting in one and the same tissue concerned with lactose metabolism has been reported by Wood. It is quite possible then that further exploration may reveal the existence of similar dual enzymatic systems engaged in other types of enzymatic reactions.

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Chapter 15

GLUCAGON AND EPINEPHRINE

Earl W. Sutherland

Glucagon and the sympathomimetic amines, epinephrine and norepinephrine, are chemical agents present in vertebrates and are capable of producing hyperglycemia. When small amounts of glucagon or epinephrine are injected intravenously into man or experimental animals, hyperglycemia appears almost immediately after injection, but it is of short duration. Although glucagon and epinephrine have a similar action in the liver, it seems advisable to consider them separately in the beginning since they differ considerably in a number of respects.

GLUCAGON

Glucagon is a small protein of known structure that is present in the pancreas and, to a variable extent, in the gastrointestinal tract. A large variety of other tissues do not contain glucagon in readily detectable amounts. The structure, distribution, and cellular origin of glucagon are discussed in detail in previous chapters, therefore, only a brief review is included here.

The concentration of glucagon is related to the amount of islet tissue present in the pancreas The tail or splenic portion of the dog pancreas

is relatively rich in islet tissue and contains much more glucagon than is found in the head of the pancreas. The fetal calf pancreas contains relatively large amounts of glucagon and islet tissue. Following pancreatic duct ligation, the sclerosed pancreatic tissue contains an increased amount of glucagon per unit weight. Extracts from the pancreas of the alloxan-diabetic rats contain normal amounts of glucagon, while insulin is greatly diminished or absent. These findings have indicated that glucagon in the islet tissue is present in other than β-cells. Numerous experiments have been devised in order to identify the cell of origin more exactly; these have led to varied conclusions regarding probable α-cell origin and are discussed in Chapter 5.

Evaluation of the physiologic or pathologic role of glucagon has been limited by lack of a method for assay of this substance in blood. Several reports have indicated the presence of materials exhibiting hyperglycemic activity in the blood of man and experimental animals; however, the relationship of these materials to glucagon has not been clearly demonstrated. References to these experiments are contained in a recent report in which a glucagon-like material was found in the blood of man and dog by Makman *et al.* A plasma fractionation procedure was developed in which glucagon was recovered in high yield and inhibitory plasma factors were removed or inactivated. Average plasma levels in man and dog were found to be about 7 μg. per 100 milliliters, which would represent nearly 0.2 mg. of the circulating plasma glucagon in a 70 kg. man. Various fractionation and characterization studies showed that this material was similar to glucagon in some respects, but more recent experiments have indicated that a considerable part of this material is not actually glucagon.

Action of Glucagon in the Intact Animal

The outstanding response to be noted after injection of glucagon is the hyperglycemia that results from an increased rate of glycogen breakdown in the liver. The hyperglycemia, therefore, is dependent on adequate amounts of liver glycogen; the mechanism producing the increased breakdown of glycogen is discussed in a later section. Studies of the hyperglycemic response to glucagon in normal or diseased states may reflect variations in the glycogen reserves rather than abnormalities of glycogenolytic mechanisms, such as may be encountered in glycogen storage diseases, for reference, see Chapter 30 and (4). If adequate amounts of glycogen are present in the liver, hyperglycemia is the classic response to glucagon in man or in various vertebrates, and the hyperglycemia is accompanied by a decrease in the amount of liver glycogen.

A "rebound" phenomenon has been observed some hours or one day after glucagon injection; the glycogen content of the liver may rise above the preinjection or control levels, according to studies by Root, Foa *et al.*, and Kalant. It seems likely that glucagon has indirectly brought about an increase of gluconeogenesis, which is noted after the primary action of glucagon has disappeared.

The effect of glucagon on the *extrahepatic utilization of glucose* has been studied in many laboratories and the results have been discussed in the reviews listed at the end of this section. Some investigators have reported an increased utilization of glucose, some a decreased utilization, while the majority report that glucagon has no effect on the extrahepatic utilization of glucose. The evidence indicates that there is no readily demonstrable effect of glucagon on glucose utilization by muscle or on glycogen breakdown by muscle. It seems likely that some early samples of glucagon contained small amounts of insulin. Blood lactate and pyruvate change very little after glucagon injection; with small or moderate doses some decreases in lactate and pyruvate levels have been reported.

Although in vitro effects of glucagon on the synthesis of fats, cholesterol, and ketone bodies in the liver have been reported and confirmed, glucagon effects on lipids in the intact animal have not been striking in general. Cavallero has reported changes in metabolism of fats in dwarf mice, and Foa and collaborators have reported increased ketonemia in depancreatized dogs. Perhaps in vivo effects of glucagon on lipids may be seen only when certain abnormalities of metabolism exist. An increased excretion of nitrogen has been reported by Tyberghein and by Kalant.

Reports of other glucagon effects have appeared recently, including the one by Staub *et al.*, stating that glucagon increases the excretion of certain inorganic ions. Izzo and Glasser have reported an increased excretion of nitrogen in fasting rats; this was not seen after epinephrine administration in their experiments. Davidson, Salter, and Best have shown an increase in oxygen consumption following glucagon injection into fed or fasted rats.

Reviews with numerous references to glucagon have been written by de Duve and Berthet (1957), Foa *et al.* (1957), Best *et al.* (1955), Bürger (1950), and by a number of participants in a Ciba Foundation Colloquia (1956).

Possible Relation to Diabetes Mellitus

Since a number of diabetics appear to have roughly normal amounts of insulin in the pancreas or plasma, investigators have studied a large

variety of factors that might explain such findings. Another point that has been considered is the possibility that one or several factors might be important in causing stress in the pancreas at some time during life. At the moment neither the physiologic nor the possible pathologic role of glucagon is known. Ingle discusses experimental diabetes in detail in a later chapter. Interest in the relation of glucagon to diabetes was stimulated by comparison of alloxan-diabetic dogs versus depancreatized dogs in Young's laboratory, and has continued in part because of reports by Salter *et al.* that glucagon has a diabetogenic effect in force-fed rats and dogs, and that when given with cortisone, a temporary "diabetic state" can be produced in rabbits by Lazarus and Volk. Numerous negative results have also been reported. Since considerable species variation exists in the experimental production of diabetes, it will be of interest to pursue the analysis in man as well as in experimental animals.

SYMPATHOMIMETIC AMINES

The sympathomimetic amines, primarily epinephrine and norepinephrine, are produced by the adrenal medulla, by postganglionic sympathetic nerve fibers, and they are also present in the central nervous system where they are most likely produced to some extent. This would appear to be an ideal system for minute to minute regulation of the blood sugar, since the secretory system is intimately connected to the central nervous system—which is most sensitive to glucose deficiency and is essential to life. Secretion of amines, primarily epinephrine, from the adrenal medulla has long been noted in hypoglycemia and in certain other conditions or situations. The sympathetic nerve fibers produce norepinephrine primarily, and it has been difficult to evaluate the significance of this production in terms of importance in the control of carbohydrate metabolism in the intact animal. Certainly stimulation of the hepatic nerves can produce a hyperglycemia.

Following an injection of epinephrine, prominent responses occur in various smooth muscles, in cardiac tissue (7), and in metabolism, especially of carbohydrates (5). The changes in carbohydrate metabolism are more complex than those resulting from the injection of glucagon. Glycogenolysis in the liver is stimulated by epinephrine as it is by glucagon and will be discussed separately. In addition, glycogen breakdown in muscle is accelerated; in this case the end product of glycogenolysis is lactic acid rather than glucose, since muscle does not have the enzyme glucose-6-phosphatase, which is present in large amount in the liver. As a result, a rise in blood lactic acid is noted along with hyperglycemia. As described by the Coris, the lactate may cir-

culate to the liver and be converted to glucose or glycogen. In addition to or coincident with the increased breakdown of glycogen in muscle, there is a decrease in glucose uptake by skeletal muscle. Again, this appears to be an excellent mechanism for protecting the central nervous system, for muscle can utilize other substrates, including ketone bodies. The exact mechanism of this decreased uptake of glucose is not clear, even though it is tempting to ascribe the decrease to the presence of increased amounts of glucose-6-phosphate in the muscle.

Increased catabolism of fat and protein has been observed frequently. Small or moderate amounts of epinephrine produce an increase in oxygen consumption, possibly related to the increased production of lactic acid. An increase in plasma potassium and a decrease in serum phosphate occur after intravenous injection of epinephrine. The relation of these events to carbohydrate metabolism is currently debated. There also seems to be a relation of epinephrine action to thyroid activity; when more thyroid hormone is present, there is a greater response to epinephrine.

The possible relation of sympathomimetic amines to diabetes mellitus is not clear in many respects. We know that epinephrine tends to antagonize the hypoglycemic activity of insulin. In the rabbit, severe hypoglycemia that would be produced by a given amount of insulin can be avoided by simultaneous subcutaneous injection of epinephrine; in fact, hyperglycemia will result if relatively larger amounts of epinephrine are given. Similar antagonism to insulin hypoglycemia has been observed in other species.

In man, epinephrine has been used to relieve insulin hypoglycemia. Emotional disturbances may cause variations in insulin requirement; the possible relation to sympathomimetic amines has not been clarified. To date, determination of catecholamines and their degradation products in plasma and urine have been unsatisfactory because of inadequate methods of analysis, or in some cases, inadequate knowledge of the degradation products. A symposium regarding catecholamines, including problems of determination, will be sponsored by the National Institutes of Health, and the papers given are to be published in *Pharmacological Reviews* in 1959

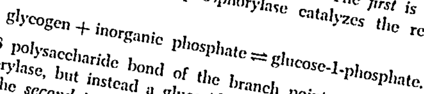
ACTION OF GLUCAGON AND EPINEPHRINE IN LIVER

Action in Liver Slices

Glucagon and epinephrine increase glycogen breakdown and glucose

glucagon and epinephrine with liver slices, and the relative activities of several sympathomimetic amines are determined. Half maximal responses are found when about 5×10^{-7} M epinephrine are added, and the relative activities of all amines tested parallel the order of hyperglycemic activity in the intact animal. These and other experiments indicate that liver slices are suitable preparations for the study of the action of glucagon and epinephrine. It is established that some phosphorolytic mechanism is stimulated, which results in an increased accumulation of glucose in liver cells, with subsequent outpouring of glucose into the medium.

Dr. Stetten has discussed previously the enzymatic mechanisms involved in the phosphorolytic conversion of glycogen to glucose in liver. In brief review, three major steps are involved. The *first* is the phosphorylase reaction in which phosphorylase catalyzes the readily reversible reaction



$$\text{glycogen} + \text{inorganic phosphate} \rightleftharpoons \text{glucose-1-phosphate.}$$

The 1-6 polysaccharide bond of the branch points is not cleaved by phosphorylase, but instead a glucosidase catalyzes a hydrolysis at this point. The *second* is the reversible conversion of glucose-1-phosphate to glucose-6-phosphate, catalyzed by phosphoglucomutase in the presence of appropriate cofactors. The *third* is the hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate, catalyzed by the glucose-6-phosphatase present in the liver microsomes. Study of metabolites within the slices has revealed that glucagon and epinephrine increase the amounts of glucose-1-phosphate and glucose-6-phosphate within the cell, thus indicating that the phosphorylase system is stimulated. Direct measurements of phosphorylase activity in homogenates from slices preincubated with glucagon or epinephrine show an increased concentration of active liver phosphorylase. (It has also been shown that epinephrine, but not glucagon, increases the concentration of phosphorylase *a* in rat diaphragm.) At this stage, it seems advisable to mention that when active phosphorylase concentration increases, there is increased glycogen breakdown, not an increased formation of glycogen. Such findings, coupled with analyses of levels of orthophosphate and hexosephosphates in various tissues, lead one to feel that phosphorylase may not be the sole or even the primary pathway to glycogen synthesis. In this connection a recent report (Leloir *et al.*) is of considerable interest, since it produces evidence for an alternate pathway of glycogen synthesis in mammalian tissue with uridine diphosphoglucose serving as the donor of hexose units in the formation of glycogen.

For some time it was not possible to establish reproducible hormonal

effects in broken cell preparations, although numerous attempts were made to increase the activity of liver phosphorylase in these preparations. A detailed study of the inactivation and reactivation of liver phosphorylase using purified enzymes was made. In brief summary, it was found that liver phosphorylase was inactivated by a phosphatase whose action resulted in the formation of an inactive form of the enzyme and inorganic phosphate. The reactivation process with donation of phosphate to the inactive enzyme was shown to occur in liver slices, and later a soluble liver enzyme (kinase) that catalyzed the activation in the presence of adenosine triphosphate and magnesium ions was found. (A similar enzyme in muscle was described by Fisher and Krebs.) The interconversion of active liver phosphorylase and inactive liver phosphorylase occurred at an extremely rapid rate; the time for interconversion of half the molecules was estimated to be about one minute. In terms of analysis of hormone action, perhaps the most important knowledge gained from these studies was that the reactivation (kinase) reaction required magnesium ions and adenosine triphosphate, and that magnesium ion concentration should exceed the adenosine triphosphate concentration. This information was most helpful in establishing reproducible hormone effects in broken cell preparations.

Effects of glucagon and epinephrine on lipid synthesis in liver slices have been demonstrated. Haugaard *et al.* and Berthet *et al.* have shown that glucagon or epinephrine inhibits the incorporation of acetate, glucose, or fructose into fatty acids synthesized by liver slices. Berthet has shown that incorporation of these substrates into cholesterol is likewise inhibited by glucagon. The relation of these effects to those of glucagon and epinephrine on phosphorylase has not been clarified. De Duve *et al.* have shown that glucagon inhibits the synthesis of hypoxanthine in pigeon liver under certain conditions, while the production of hypoxanthine from purines did not appear to be affected.

Action of Glucagon and Epinephrine in Broken Cell Preparations

When liver homogenates were incubated with suitable amounts of magnesium ions and adenosine triphosphate, it was possible to show large and reproducible increases in accumulation of liver phosphorylase following addition of glucagon or epinephrine. Phosphorylase reactivation, in reaction mixtures of 0.2 ml. or less, was stimulated by 1×10^{-7} M epinephrine, or by about 1×10^{-8} M glucagon, thus allowing detection and assay of small fractions of micrograms of either hormone. The relative activities of a number of sympathomimetic amines were the same when determined by homogenate assay, by liver slice assay, or by assay in intact animals. Moreover, the action of epinephrine

was blocked by ergotamine, while the action of glucagon was not, thus allowing assay of glucagon in the presence of epinephrine.

The response of the homogenate to glucagon or epinephrine was separable into two phases. In the first phase, a particulate fraction from cells produced a heat-stable factor, i.e., washed particles in the presence of magnesium ions, adenosine triphosphate, and glucagon, or epinephrine produced a very stable compound, which did not contain glucagon or epinephrine. In the second phase, this factor stimulated the activation of phosphorylase in supernatant fractions where glucagon and epinephrine had no effect. *Therefore, the basic action of glucagon and epinephrine was to promote the accumulation of a stable compound in the presence of cell particles, and this compound, not glucagon or epinephrine, was responsible for the enzyme activation.*

The heat-stable factor has been isolated from large-scale particle reaction mixtures by ion exchange chromatography followed by subsequent crystallization. Accumulation of this adenine ribonucleotide was increased by the addition of caffeine to the reaction mixture because caffeine inhibited the phosphodiesterase, which in turn inactivated the nucleotide. This enzyme is present in a number of animal tissues and is especially active in the central nervous system. When the adenine ribonucleotide was incubated with this enzyme, it was quantitatively converted to adenosine-5-phosphate. When hydrolysis of the ribonucleotide was catalyzed by the hydrogen form of Dowex-50, the products were identified as adenine and a mixture of ribose-3-phosphate and ribose-2-phosphate. All evidence indicated that the compound was a cyclic adenylic acid and it was found to be identical to the cyclic adenylic acid isolated by Cook, Lipkin, and Markham from barium hydroxide digest of adenosine triphosphate. In an early note, these authors felt that the compound most likely was a diadenylic acid, but Dr. Lipkin has since announced that the compound is a monomer and, therefore, adenosine-3′:5′-phosphate.

An assay for minute amounts of cyclic adenylic acid has been developed with use of liver homogenate supernatant fractions.

The cyclic adenylic acid-forming activity associated with the particulate fractions resides in the nuclear fraction, and while labile under many conditions, it is extremely stable in others. Activity is present after vigorous blendorization in hypotonic solutions and after freezing, treatment with triton, extraction with concentrated salt solutions, and other procedures that obliterate usual cell structures. To date, the response to hormones has remained even in association with the severely traumatized and partially purified particles. The origin of the active particulate material in the nuclear fraction is unknown; nuclei or cell membranes have been considered the most likely source.

PRODUCTION OF CYCLIC ADENYLIC ACID IN NONHEPATIC TISSUE

In the preceding section it was noted that the basic action of glucagon and epinephrine in the liver was to promote the accumulation of cyclic-3,5-adenylic acid. Several other tissues have been examined for their ability to form cyclic adenylic acid and some experiments with glucagon and epinephrine have been made. Particulate preparations from heart, skeletal muscle, brain, kidney, and chicken blood formed cyclic adenylic acid under the conditions used for liver. In none of these preparations has a stimulation by glucagon been noted, although epinephrine markedly stimulated the accumulation in heart or skeletal muscle preparations. For reference to phosphorylase activation and cyclic adenylic acid production, see (9) and (10).

An extremely interesting study of the action of adrenocorticotrophic hormone (ACTH) by Haynes (8) has led to the conclusion that ACTH activates phosphorylase in adrenal gland slices and that it produces an increased amount of cyclic adenylic acid in adrenal slices. He proposes that ACTH (but not glucagon or epinephrine) increases the concentration of cyclic adenylic acid in the adrenals which, in turn, activates phosphorylase. The increased amount of phosphorylase increases glycogenolysis with a resultant increase of glucose-6-phosphate. The powerful glucose-6-phosphate dehydrogenase of the adrenal glands catalyzes the oxidation of this substrate, with concomitant production of reduced triphosphopyridine nucleotide, thus accelerating steroid production.

It seems that three hormones which increase blood sugar act through a common mediator, i.e., cyclic-3,5-adenylic acid. The "receptor" varies from organ to organ, and in each case requires a specific hormone, except in the liver, where either glucagon or epinephrine is active, although perhaps at different sites. The end product also varies from organ to organ, with glucose production occurring in liver, lactate production in muscle, and steroid production occurring in the adrenal gland. Although extension of these findings may not reveal any close association with diabetes mellitus, it seems that a number of interesting areas invite further exploration.

SUMMARY

Although its physiologic and possible pathologic roles are still unknown, glucagon is able to cause hyperglycemia by promoting breakdown of liver glycogen and, under certain conditions, can reduce or eliminate an insulin hypoglycemia. The production of hyperglycemia from breakdown of liver glycogen is the outstanding response to glu-

cagon injection, but various other effects of glucagon have been described, which may not be related to the action on liver glycogen. A glucagon-like material has been measured in plasma of man and dog, but a considerable portion of this material was not glucagon; ability to measure glucagon in plasma may permit further evaluation of its role in man. The action of epinephrine on carbohydrate metabolism is more complex, since epinephrine also increases glycogen breakdown in certain nonhepatic tissues, including skeletal muscle, and decreases the glucose uptake by skeletal muscle.

Glucagon and epinephrine increase the concentration of active phosphorylase in liver. The concentration of active phosphorylase represents a balance between inactivation by a phosphatase and a reactivation by a kinase. Glucagon and epinephrine displace this balance in favor of the active phosphorylase. The basic action of glucagon and epinephrine is to promote the accumulation of cyclic-3,5-adenylic acid and this compound, not glucagon or epinephrine, is responsible for the increased conversion of inactive to active phosphorylase. Epinephrine, but not glucagon, increases the accumulation of this cyclic nucleotide in preparations from heart and skeletal muscle.

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Chapter 16

METABOLIC EFFECTS OF ADRENAL CORTICOSTEROIDS*

Albert E. Renold and James Ashmore

INTRODUCTION

The probable existence of a direct adrenal influence upon carbohydrate metabolism was first suggested by Bierry and Malloizel's observation, 1908, of hypoglycemia in adrenalectomized dogs and Porges' independent description, 1909, of fasting hypoglycemia in patients with Addison's disease. Following the discovery of insulin, Maranon reported in 1925 that patients with adrenal insufficiency exhibited marked sensitivity to insulin and, shortly thereafter, the existence of diminished hepatic glycogen reserves in fasted adrenalectomized animals was conclusively established by Cori. Between 1934 and 1936, the striking amelioration of the diabetic state that follows adrenalectomy was suggested (Hartman, 1934) and established (Long and Lukens, 1935 and 1936). The then prevailing knowledge was analyzed with particular clarity by Britton and Silvette in 1932, and in a now classic report by Long, Katzin, and Fry (1940). To the latter investiga-

* Supported in part by grants from the Adler Foundation, Inc., Rye, New York and Eli Lilly and Company, Indianapolis, Indiana

tors it appeared established in 1940 (a) that *fasted*, adrenalectomized animals show decreased levels of blood glucose as well as of liver and muscle glycogen, whereas *fed* adrenalectomized animals maintained on saline do not; (b) that the administration of adrenal cortical extract results in increased levels of blood glucose and liver glycogen and increased nitrogen excretion; (c) that these observations could best be interpreted as indicating that adrenalectomized animals are unable to synthesize glucose from endogenous protein sources at a rate sufficient to prevent liver glycogen depletion and hypoglycemia under fasting conditions.*

The analysis of Long, Katzin, and Fry still covers the major portion of what is securely established today. In trying to review the present state of knowledge the authors will in the main extend these observations to information that has resulted from the development of new tools and techniques: isolated tissue preparations and the availability of isotopically labeled substrates, isotopic dilution techniques, improved methods for purifying and measuring enzymatic activities, and pure hormone preparations available in convenient form, to mention but a few. In so doing some emphasis will be given to biochemical sequences *in time*, in an attempt to evaluate the relation of each metabolic event to the postulated (and as yet unknown) primary site of glucocorticoid action.

STEROID STRUCTURE AND GLUCOCORTICOID ACTIVITY

The term glucocorticoid, introduced by Selye, still seems the most adequate one to define that type of adrenal cortical steroid which exerts a major influence upon intermediary metabolism of mammalian cells. Even if a substance exhibiting such activity is not of adrenal origin, it should be termed a substance with glucocorticoid activity. If one considers adrenal cortical steroids as structurally derived from progesterone (21-carbon chain, 4–5 unsaturation, ketone substitutions in position 3 and 20) then it would appear that hydroxylation in position 21 generally increases the biologic activity of the steroid (both with regard to electrolyte activity and to activity on intermediary metabolism) whereas hydroxylation in position 11 or 17 mainly increases glucocorticoid activity. In position 11, the oxygenated derivative may be either the alcohol or the ketone The major naturally occurring glucocorticoids are 11-dehydrocorticosterone, corticosterone; 17-hydroxy, 11-dehydrocorticosterone (cortisone); and 17-hydroxycorticosterone (cortisol) in order

* For historical references listed here, see Thorn *et al.*, 1957.

of increasing potency. It is of great interest that organic chemists have *succeeded in synthesizing* several further substances with glucocorticoid activity by making substitutions within the molecule which are as yet not known to occur biologically. In Table 16-1 an attempt has been

TABLE 16-1. GLUCOCORTICOID AND SODIUM-RETAINING ACTIVITY OF SOME NATURALLY OCCURRING AND SYNTHETIC STEROIDS (POTENCY OF CORTISOL = 1)

Compound	*Major difference from cortisol*	*Activity*	
		Glucocorticoid	*Na-retaining*
Desoxycorticosterone acetate	$-11\beta OH$	<0.02	30-50
Corticosterone	$-17\alpha OH$	0.2	1
Cortisol		1	1
Cortisone		.8-1	.8-1
Prednisolone	$+\Delta$ 1-2	3-5	slight
Fluorocortisol	$+9\alpha F$	15-20	200-800
6-Methylprednisolone	$+\Delta$ 1-2, $+6\alpha CH_3$	3-5	slight
16-Hydroxyfluoroprednisolone	$+\Delta$ 1-2, $+9\alpha F$, $+16\alpha OH$	4-6	slight
Fluoroprednisolone	$+\Delta$ 1-2, $+9\alpha F$	30-40	200-800

made to compare the glucocorticoid and sodium-retaining activity (*milligram per milligram*) *of some natural and synthetic steroids with* glucocorticoid activity, the activity of cortisol being taken as unity. The table is based on information gathered from studies in animals as well as in man, although preference has been given to studies in man. Other estimates have been recently assembled and edited by Soffer and Orr.

GLUCOCORTICOID EFFECTS UPON CARBOHYDRATE BALANCE

Observations on Whole Animals

That glucocorticoids, when administered to the intact and fasting mammalian organism, produce increased hepatic glycogen stores and increased levels of blood glucose (as illustrated in Fig. 16-1), is generally accepted. Since the total carbohydrate stores of glucocorticoid-treated fasting animals increase, and since urinary nitrogen excretion increases simultaneously, increased gluconeogenesis from protein is one likely and widely accepted mechanism for the occurrence of these effects. Entirely consonant with these *findings* and their *interpretation*

are the decreased hepatic and muscular glycogen stores, associated with lower than normal blood glucose levels, characteristic of adrenalectomized animals subjected to even relatively short periods of fasting. Equally compatible are the markedly lessened glucosuria and hyperglycemia of

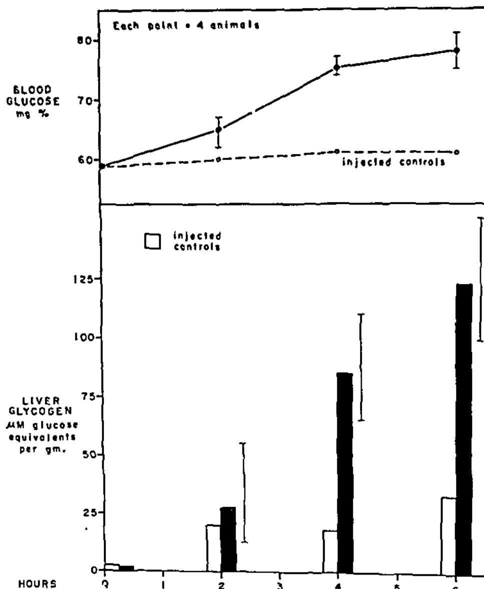


FIG. 16-1. Effects of cortisol (5 mg. subcutaneously) on blood glucose and liver glycogen of normal fasted rats.

diabetic animals after induction of adrenal cortical insufficiency (Chap. 18).

More direct testing of the hypothesis that glucocorticoids enhance gluconeogenesis, and therefore increase hepatic glucose output, has come

from studies of Welt, Stetten, Ingle, and Morley in rats and of Altszuler, Steele, Wall, and deBodo in dogs. Using glucose labeled with C^{14} to identify glucose already present in the extracellular body pool at the beginning of any experimental period, and decreasing specific activity of this glucose as a measure of hepatic production of newly formed (unlabeled) glucose, both groups of investigators have demonstrated beyond reasonable doubt that the administration of glucocorticoids results in a markedly increased hepatic production of blood glucose, while hypoadrenocorticism is accompanied by decreased hepatic glucose production.

Since glucose synthesis from fat almost certainly does not occur to any significant extent in the mammalian organism, one would reasonably expect gluconeogenesis from protein to account for the increased hepatic synthesis of glucose and glycogen induced by glucocorticoid administration. However, Ingle and Thorn, in partially pancreatectomized rats given cortisone, and Ingle, in force-fed cortisol-treated rats, found that the increased nitrogen excretion produced by the glucocorticoids was inadequate to account for the concurrent increase in glucose excretion. This discrepancy suggested an added glucocorticoid effect upon carbohydrate balance: decreased extrahepatic glucose utilization. The presence or absence of such a glucocorticoid effect resulting in decreased glucose utilization has been the subject of considerable controversy and will not be debated here, since an excellent and extensive review of the available information has been recently presented by deBodo and Altszuler. The authors of this chapter interpret the evidence available to them as failing definitively to support the concept of decreased glucose utilization by extrahepatic tissues as a result of glucocorticoid action. However, studies from Long's laboratory (with Winternitz and Dintzis) clearly suggest that glucocorticoids may decrease *hepatic* glucose utilization in the intact animal Furthermore, these studies also suggest an important role of the adrenal cortex in controlling the metabolic fate of various substrates after their uptake by the liver cell. Thus the liver of adrenalectomized animals was unable normally to synthesize glycogen from glucose, fructose, glycerol, or malate, while its ability to produce glucose from glycerol, lactate, or malate was less impaired. The total amount of "glycogen and glucose" synthesized from these compounds was, of course, less than normal.

Observations on Isolated Tissue

Further support for the gluconeogenetic action of glucocorticoids comes from studies in isolated tissues, mostly rat liver slices. Since failure to demonstrate significant effects of crystalline glucocorticoids added

directly to tissue preparations in vitro has been almost universal, the majority of the observations on which this discussion is based have been made on tissues isolated from animals subjected to endocrine manipulation days or hours prior to sacrifice. A number of these studies are summarized in Table 16-2, using glucose synthesis from pyruvate as meta-

TABLE 16-2. OVER-ALL GLUCOSE PHOSPHORYLATION AND SYNTHESIS OF NEW GLUCOSE FROM PYRUVATE BY LIVER SLICES OBTAINED FROM FED RATS IN VARIOUS ENDOCRINE STATES
(Data from References 2, 3, 28)

Endocrine state	*Mean glucose phosphorylation μM/Gm. liver/90 minutes*	*Gluconeogenesis from pyruvate μM/Gm. liver/90 minutes*
Normal	42	16
Diabetic	10	48
Adrenalectomized	29	15
Diabetic-adrenalectomized	9	22
Cortisol-treated normal	36	—*
Cortisol-treated diabetic-adrenalectomized	not measured	50

* Variable although increased over-all glucose production has been established (Reference 29).

bolic indicator of gluconeogenesis, and over-all glucose phosphorylation as an indicator of glucose utilization. The liver slices were obtained from normal, diabetic, adrenalectomized, diabetic-adrenalectomized, cortisone-treated normal, and cortisone-treated diabetic-adrenalectomized rats. It is evident that while adrenalectomy alone did not significantly influence glucose synthesis from pyruvate in isolated liver, adrenalectomy superimposed upon pre-existing diabetes almost returned to normal the characteristically excessive gluconeogenesis of diabetic liver. Cortisone administration, on the other hand, resulted in markedly increased hepatic glucose synthesis from pyruvate both in normal and in diabetic-adrenalectomized animals. In contrast, adrenalectomy or cortisone administration failed to influence glucose phosphorylation by liver slices obtained either from pretreated normal or pretreated diabetic animals.

Most of the enzymatic reactions of glycolysis are freely reversible. There are, however, three notable exceptions: (1) Glucose phosphorylation is catalyzed by hexokinase, while its dephosphorylation is catalyzed by glucose-6-phosphatase. (2) Fructose-6-phosphate is further phosphorylated to fructose-1,6-diphosphate in the presence of phosphofructokinase while the reverse reaction is catalyzed by the enzyme fructose

diphosphatase. (3) Although phosphoenolpyruvate is freely metabolizable to pyruvate, pyruvate cannot be reconverted to phosphoenolpyruvate directly but only by way of malate (with incorporation of CO_2), oxaloacetate, and subsequent decarboxylation to phosphoenolpyruvate. As particularly emphasized recently by Krebs, these three steps, providing for separate reactions "from right to left and from left to right," would *a priori* seem to be particularly suitable sites for the exertion of a *directional* control of metabolic flow, since they provide *directional* flow valves. It is therefore of considerable interest to find selectively increased glucose-6-phosphatase activity (Ashmore *et al.*, 1956, Weber *et al.*) increased fructose diphosphatase activity (Mokrasch *et al.*) and increased incorporation of CO_2 into hepatic glycogen and glucose (Ashmore and Mahler) resulting from cortisol overdosage in rats.

Although it would be tempting to speculate that a direct glucocorticoid effect might be exerted at one of these points, Ashmore *et al.* (1956) have pointed out for one of them (glucose-6-phosphatase) that blood glucose and liver glycogen changes resulting from cortisone administration *precede* (rather than follow) alterations in glucose-6-phosphatase activity, strongly suggesting that the enzymatic change is of a secondary adaptive nature. These time relationships are also illustrated further on in Figure 16-6.

Whether glucose utilization by isolated extrahepatic tissues is affected by glucocorticoid action is again (as in the case of the whole animal) unclear. Whereas isolated rat diaphragm muscle from adrenalectomized and diabetic-adrenalectomized animals appears to "take up" more glucose than diaphragm from normal and diabetic animals, respectively, an effect of glucocorticoid administration has not been demonstrated. The evidence obtained from studies on eviscerated or hepatectomized preparations is equally contradictory on this point.

Observations in Man

With regard to glucocorticoid effects on glucose balance, information available in man, although more indirect in nature, agrees remarkably well with the information available in intact animals and their isolated tissues. Patients with Addison's disease exhibit fasting hypoglycemia and subnormal urinary nitrogen excretion while glucocorticoid administration increases fasting levels of blood glucose as well as the urinary excretion of nitrogen. Furthermore, rather direct evidence for the gluconeogenetic effects of glucocorticoids in the intact organism has been obtained, in the opinion of the authors of this chapter, from observations in two fasting subjects with renal glucosuria, observations

illustrated in Figure 16-2. In both instances, cortisol was administered intravenously over eight hours, the subjects having been previously fasted for 16 hours and continuing to fast throughout the experimental period. In both instances urinary glucose excretion increased within two

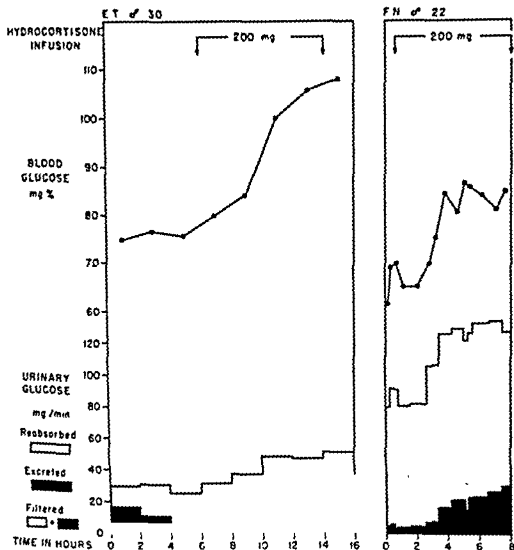


Fig 16-2. Effects of cortisol on blood glucose and renal glucose clearance in 2 fasted patients with renal glucosuria. (From Froesch, Winegrad, Renold and Thorn, reference 10)

hours of the beginning of cortisol administration and reached maximal rates three and eight times greater than the initial one. Despite this considerably increased urinary glucose loss during fasting, blood glucose levels significantly increased in both subjects. Since it may be safely assumed that in fasting individuals given cortisol liver glycogen is in-

creasing and not decreasing, and since in man glucose utilization by tissues such as muscle is known to decrease to almost unmeasurable levels after 16 hours of fasting, the most reasonable conclusion which these observations allow *is that of increased hepatic gluconeogenesis of* the order of magnitude of several grams per hour as a result of cortisol administration. Further evidence obtained in man has been recently reviewed (Thorn *et al.*).

It is necessary to point out that recent observations on blood pyruvate, lactate, and α-ketoglutarate in normal subjects, in patients with Cushing's syndrome, and in patients undergoing high-dosage glucocorticoid therapy, have established the interesting fact that moderately elevated levels of blood lactate and pyruvate are a characteristic feature of spontaneous or induced hyperadrenocorticism (Frawley, Hennes, *et al.*). In addition, these observations have been interpreted as indicative of a decreased ability to metabolize pyruvate and it has even been suggested that glucocorticoids exert a direct inhibitory effect upon the oxidative decarboxylation of pyruvate in tissues. This interpretation is as yet highly speculative.

In man, as in experimental animals, the evidence relating to a glucocorticoid effect on glucose utilization is as yet contradictory. It has been reviewed by deBodo and Altszuler.

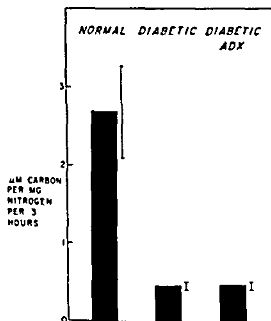
GLUCOCORTICOID EFFECTS UPON LIPID METABOLISM

Alterations of lipid metabolism resulting from altered states of adrenal cortical function have been reported. Perhaps the most striking change is the markedly *depressed hepatic synthesis of long-chain fatty acids* observed by Brady, Lukens, and Gurin after pretreating rats with cortisone. Furthermore, the impaired lipogenesis characteristic of liver tissue obtained from diabetic animals is returned almost to normal by adrenalectomy, suggesting a possible direct adrenal cortical effect upon lipogenetic pathways. Recently, however, Jeanrenaud *et al* have established that this glucocorticoid effect is limited to liver, and cannot be demonstrated upon fatty acid synthesis by adipose tissue, a more highly specialized and somewhat less complex tissue in this regard. When both hepatic and adipose tissue lipogenesis from pyruvate are compared in tissues obtained from the same normal, diabetic, and diabetic-adrenalectomized animals (Fig. 16-3) it is evident that alloxan diabetes is accompanied by markedly inhibited lipogenesis in both tissues, while superimposed adrenalectomy returned only hepatic lipogenesis toward normal. These findings suggest that the glucocorticoid effect observed upon hepatic lipogenesis alone may well be *secondarily* related to other

metabolic effects of the hormone in this tissue, such as, for instance, the reversal of glycolysis resulting from increased hepatic gluconeogenesis. Further observations on the sequence of biochemical events in time similarly indicate that the glucocorticoid effect on hepatic lipogenesis is unlikely to represent a primary site of glucocorticoid action. Finally, the inhibitory corticoid effect upon lipogenesis would appear to be

LIPOGENESIS FROM PYRUVATE-2-C^{14}:

BY RAT ADIPOSE TISSUE



BY RAT LIVER SLICES

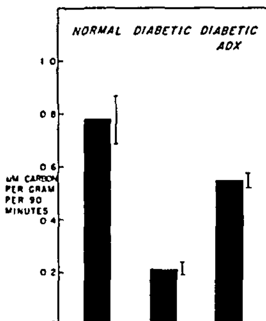


FIG. 16-3. Comparison of hepatic lipogenesis with adipose tissue lipogenesis in normal, diabetic, and diabetic-adrenalectomized rats. All values expressed as micromoles of carbon-2 of pyruvate incorporated into long-chain fatty acids. Because of differences in the reference measurements only the relative not the absolute values should be compared.

limited to certain conditions and particularly to certain species, since in the guinea pig, for instance, glucocorticoid administration results in increased rather than decreased peripheral fatty acid synthesis (Hausberger). In man, Cushing's syndrome is characterized neither by excessive nor by insufficient fat stores, but rather by *redistribution* of storage lipids and by a decreased protein to fat *ratio*.

Whereas a direct glucocorticoid effect upon fatty acid synthesis appears unlikely, at this time, and to these observers, fatty acid mobilization and oxidation may be a more promising site for a possible direct

effect of glucocorticoids upon lipid metabolism. Scow has recently re-emphasized the ketogenetic activity of cortisone and demonstrated a dramatic effect of this hormone upon blood ketone levels in pancreatectomized rats. The mechanism of fatty acid or triglyceride release from adipose tissue is presently under close scrutiny in several laboratories and may yet prove to be a primary site of metabolic adrenal cortical effectiveness. The endocrine control of ketogenesis has been reviewed by Engel.

The following reasoning, *clearly labeled pure speculation,* may be of interest: Since increased gluconeogenesis results from glucocorticoid action by quite general consent; since it does not seem possible to account for all the excess glucose on the basis of increased gluconeogenesis from protein; and since gluconeogenesis from fat does not occur; then would not increased mobilization and oxidation of fat permit the utilization of larger numbers of three-carbon fragments for the resynthesis of glucose, without inhibition of glucose utilization per se? Indeed, this increased oxidation of fats would be directly useful in supplying the additional hydrogen required for the resynthesis of glucose from three-carbon fragments. Finally, the extra glucose so resynthesized would not be expected to be accompanied by increased nitrogen excretion.

GLUCOCORTICOID EFFECTS UPON THE METABOLISM OF PROTEIN AND AMINO ACIDS

When considering adrenal cortical effects upon protein metabolism, including growth, it is necessary to hold apart the effects of small, "maintenance" doses of the hormone (differentiating the euadrenal from the hypoadrenal organism), and the effects of large, excessive amounts. Russell and Wilhelmi have recently concluded from a review of the available data that "It is evident that if electrolyte balance and nutritional status of the animal are maintained, the adrenal cortex is not required for normal growth The anabolic effects of small or moderate amounts of adrenocortical hormones in adrenalectomized animals are very likely wholly explicable as indirect effects related to hemodynamic state, general health and nutrition "

In contrast, general agreement exists with regard to the catabolic effect of large doses of adrenal cortical hormones, more specifically of the glucocorticosteroids. At higher dose levels, all substances that exhibit glucocorticoid activity also manifest (insofar as they have been tested) ability to inhibit growth, to produce negative nitrogen balance, and to lead to evident protein depletion of tissues, as in bone (osteo-

porosis), muscle (partial atrophy), skin (striae of Cushing's syndrome), and, perhaps more specifically although not better understood, lymphatic tissue (atrophy). It is of interest that in some special instances, as in the case of the uterine growth induced by estrogens, a direct antagonism between steroids has been demonstrated at the tissue level. In general, however, *the specific mechanism of glucocorticoid-induced inhibition of growth is shrouded in darkness.*

In view of the demonstrated effects of glucocorticoids upon gluconeogenesis, which have already been discussed, it has been generally tempting to link these effects with the catabolic effects of the hormones

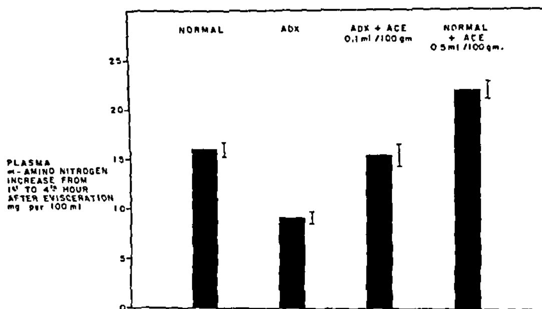


FIG. 16-4. Release of amino acids in eviscerated rats (data of Bondy, reference 6).

and to search for mechanisms that would either make increased gluconeogenesis dependent upon increased protein breakdown or increased protein breakdown dependent upon the simultaneous occurrence of increased gluconeogenesis. Since gluconeogenesis *is for practical* purposes limited to the liver, work in this area has particularly benefited from observations made under conditions allowing for at least partial functional separation of hepatic from general metabolic processes. Ingle *et al.* and Bondy have established that adrenalectomy decreases, and that administration of adrenal extracts increases, the amino acid concentration in the serum of hepatectomized animals (Fig. 16-4). Kline reported that adrenalectomy decreases the release of aminonitrogen by isolated rat diaphragm and that prior treatment of the animal with

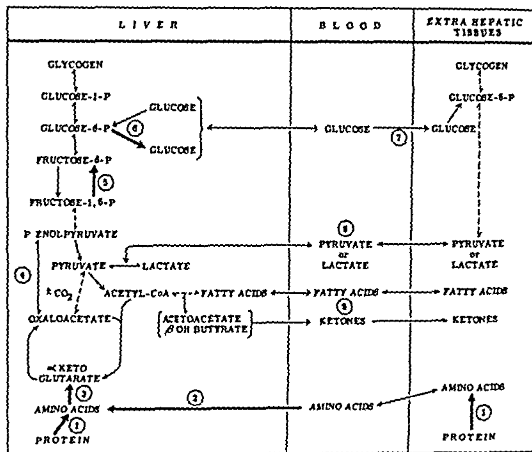


FIG. 16-5. Summary of the metabolic changes associated with glucocorticoid action which have been emphasized in this chapter. The numbers refer to the following *reactions or reaction sequences:* (1) *Increased catabolism of protein to amino acids;* (2) *Increased hepatic "trapping" of amino acids;* (3) *Increased* hepatic transamination and, for certain amino acids, deamination, (4) Increased hepatic CO_2 fixation, *suggesting increased resynthesis of P-enolpyruvate from pyruvate by way of malate and oxaloacetate;* (5) *Increased hepatic fructose-diphosphatase* activity; (6) *Increased glucose-6-phosphatase* activity; (4 + 5 + 6) Increased reverse glycolysis, i.e. hepatic gluconeogenesis; (7) Direct (controversial) or *indirect* (over-all) decrease in peripheral glucose utilization; (8) Elevated levels of blood pyruvate and lactate; (9) *Increased fatty acid mobilization and ketogenesis (both controversial).*

observations made. However, the numbering in no way indicates an established sequence of events. Which (if any), of the reactions (numbered or unnumbered) of the scheme may be regarded as the driving force of the sequence of reactions altered by glucocorticoid action is unknown. The authors would hazard a guess (an educated guess, they hope, but clearly a guess) that it might be reaction 1. The well-known discrepancy between excess glucose formed, on the one hand, and in-

creased nitrogen excretion, on the other hand, is the most frequently emphasized objection to a primary glucocorticoid effect upon gluconeogenesis. Although this objection is a weighty one, since protein is the most likely source of extra glucose, it should be pointed out that the studies upon which this objection is based are mostly short-term ones. Under these conditions a number of unknown factors could become operative and some of these, all hypothetic, should be mentioned. If the protein used for gluconeogenesis were mucoprotein in nature, a greater than expected glucose to nitrogen ratio would result. If nitrogen-sparing mechanisms (such as the formation of diamino acids or amines) exist, they might be expected to function during increased glucocorticoid activity and "stress." If three-carbon fragments resulting from *carbohydrate* metabolism are increasingly used for glucose resynthesis, increased gluconeogenesis and *apparent* decreased glucose utilization would result.

Next, since the factor *time* was mentioned in the introduction, what information can be derived from present knowledge concerning the sequence of biochemical events that follow glucocorticoid administration or withdrawal? The time sequence of some events has been shown in Figures 16-1 and 16-2, and is again illustrated in Figure 16-6. In this last figure fasting blood glucose changes reflect the most commonly noted biologic effect in animals and man, glucose synthesis from pyruvate by rat liver slices represents one of our most direct indexes of the activity of hepatic gluconeogenesis, and glucose-6-phosphatase activity has been charted as representative of the several enzymatic activities whose alteration has been reported as a result of glucocorticoid administration or withdrawal. As previously indicated, at least this enzymatic change (glucose-6-phosphatase activity) appears to *follow* functional changes and must be considered as secondary and adaptive. Quite generally, it would seem that between one and two hours have to elapse after the onset of glucocorticoid administration before any demonstrable metabolic changes are elicited. (It may be of interest to note that 1 to 2 hours is also the time required to elicit subjective improvement in a patient with adrenocortical insufficiency, given cortisol intravenously.) What happens during these 1 to 2 hours? Is this time required to set the protein catabolism or the amino acid trapping in motion? Or should we be searching elsewhere? As previously indicated, such glucocorticoid effects as the suppression of ACTH release by the pituitary occur almost instantaneously. Finally, in this regard, it should be noted that Hyde and Glenn *et al.* have investigated the fate of cortisol-4-C^{14} in rats and related its appearance in and disappearance from tissues to several biologic effects. Surprisingly, 90 per cent of the hormone had already disappeared from most tissues (and particularly

from liver) at the time of beginning, and particularly at the time of peak biologic effectiveness. This time relationship is shown in Figure 16-7. It would seem at this time that information obtained from time sequence studies strongly suggests that most or all reactions summarized in Figure 16-5 are *secondary* effects of glucocorticoid action and that

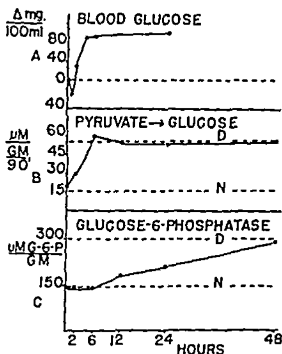


FIG. 16-6. Biochemical sequence of events after cortisol administration to diabetic-adrenalectomized rats. Curve *A*, change in blood glucose from time zero in mg. per 100 ml Curve *B*, values expressed in micromoles of pyruvate-2-C" incorporated into glucose per gram of liver per 90 minutes. Curve *C*, rat liver glucose-6-phosphatase activity as micromoles of G-6-P split per gram per 30 minutes at 30° *N*, mean normal value, *D*, mean diabetic value (From Ashmore *et al*, 1956)

they might not come very close to ... ...nary site of action of these hormones.

In the section d... ...corticoids upon carbohydrate metabolism, ... m... been made of the effect of these substances ... ...creased sensitivity

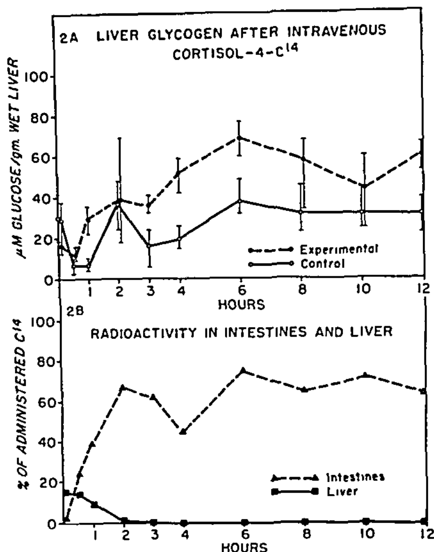


FIG. 16-7. Response and distribution of C^{14} following intravenous administration of 3 mg. cortisol-4-C^{14}/kg. Each liver glycogen value shown in *2A* is the average for 3 animals, the range being within brackets. *2B* represents C^{14} found in livers and in intestinal tracts of the corresponding animals (From Hyde, 1957.)

to insulin of the adrenalectomized organism is a generally accepted fact and has been described as early as 1925, three years after insulin became available for therapeutic use. It is also rather widely accepted that cortisone administration corrects this hypersensitive state, and that glucocorticoid overdosage results in decreased sensitivity to the hypoglycemic action of insulin, at least in many instances. Although increased hepatic glucose output may be in part responsible for the decreased sensitivity to insulin produced by glucocorticoid administra-

tion, additional factors may be involved. A direct antagonism, however, at the most likely site of insulin action, i.e., glucose uptake by tissues such as muscle, should almost certainly not be assumed. In a recent and excellent review of this topic by deBodo and Altszuler, the suggestion is made that the decreased sensitivity to insulin of the glucocorticoid-treated organism might be related to chronic hyperinsulinism (secondary to the hyperglycemia of hyperadrenocorticism) with decreased response to comparatively small additional amounts of exogenous insulin. Endogenous hyperinsulinism during prolonged hyperadrenocortical states has, indeed, been strongly indicated by Hausberger's observations in cortisone-treated guinea pigs, and suggested in man by, among others, Bastenie.

Finally, it would seem appropriate to point out—as is done more extensively in Chapter 23—that many of the differences observed when comparing euadrenal, hypoadrenal, and hyperadrenal organisms, may not be due to a direct or indirect glucocorticoid effect per se, but rather to differences in the *responsiveness* of these differently conditioned tissues. Ingle has gathered evidence to show that this is indeed the case and has termed this type of glucocorticoid action a *permissive* one. This concept, while applicable to many hormones, is particularly useful in the case of the glucocorticosteroids, since it suggests a rational approach to the nature of adrenal cortical effectiveness in increasing resistance to stress in its multiple forms. Animals suffering from adrenal cortical insufficiency show markedly decreased resistance to such varied stresses as changes in environmental temperature, trauma, infection, toxic substances of many types, exercise, fasting, and so on. It is difficult to imagine a single glucocorticoid-induced response that would be the suitable response in all these instances, unless the glucocorticoid-induced effect were one of facilitating the ability to respond as such, even though the nature of the response were different in each case. In closing, it may be permissible to ask whether a primary glucocorticoid effect, resulting in a rapid increase in the amino acid pool at the expense of protein, might not be the most appropriate response to insure adequate correction of stress-induced damage, by making available building blocks not only for the reconstitution of damaged structures, but also for the *ad hoc* "retooling" of the cell in the form of appropriate enzyme synthesis as dictated by substrate availability and need.

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Chapter 17

GROWTH HORMONE

F. G. Young and A. Korner

More than thirty years ago H. M. Evans declared his belief that the striking influence on body growth exerted by the anterior pituitary gland is mediated by the secretion of a single growth hormone by the anterior pituitary tissue, and this idea is now widely accepted. From ox pituitary glands, growth hormone (STH) was first isolated by Li, Evans, and Simpson as an apparently homogeneous protein, while later Wilhelmi, Fishman, and Russell described a means of obtaining a crystalline preparation of the hormone from ox pituitary glands in high yield. In 1952 Raben and Westermeyer developed a method quite different in principle for preparing growth-promoting extracts from pig pituitary glands, but there is evidence that this method yields inhomogeneous material (Li and Papkoff 1953).

SPECIES DIFFERENCES IN GROWTH HORMONE

Chemical and biologic differences are known to exist between the growth hormones obtained from a number of species of animal. For instance, the growth hormone recently prepared from the pituitary glands of man and monkey (Li and Papkoff, 1956, Raben, Papkoff and Li), has physical and chemical properties very different from those of

the ox hormone (*Li*). Growth hormone has also been prepared from the pituitary gland of the whale, and there is evidence for the existence of growth hormone in pituitary extracts from a number of fish. Here again species differences almost certainly exist (see Ketterer, Randle, and Young for references).

Growth hormone is believed to be produced by the acidophil cells of the anterior lobe of the pituitary gland, and the hormone has not been obtained from tissues other than those of the pituitary gland. The amount of the hormone secreted daily under normal conditions is unknown. Gemzell and Li found that single human pituitary glands contained 2.7–5.6 mg. of growth hormone. The suitable administration of 100–200 µg. of ox growth hormone daily to hypophysectomized rats, or about 5 to 10 times this daily dose into normal adult female rats, will cause an increase in body weight at an approximately maximal value.

GROWTH HORMONE AND METABOLISM

The many metabolic processes influenced by growth hormone have been reviewed recently (Smith, Gaebler, and Long; Ketterer, Randle, and Young; de Bodo and Altszuler) and they will not be discussed in detail here. Although the secretion or administration of growth hormone is necessary for growth during the early part of life in many species of animal, certain very young offspring, including those of man, can increase in weight at the normal rate for some time after birth in the complete absence of the anterior pituitary lobe. Later, the increase in weight ceases in the absence of growth hormone. When growth hormone is administered to normal or to hypophysectomized animals, the rise in body weight is accompanied by a deposition of protein, of carbohydrate, and of water, and a loss of body fat. The material laid down in the tissues under the influence of growth hormone has a composition similar to that of muscle tissue. The gain in body weight brought about by treatment with growth hormone in hypophysectomized rats is accompanied by growth of the viscera, by skeletal development, and an increase in weight of the muscles. It has been said that the composition of the body of the growth-hormone treated animal retains a juvenile pattern, with much protein and relatively little fat.

It is probable that the enhancement of protein deposition brought about by growth hormone is the result of a stimulation of protein synthesis rather than of an inhibition of the catabolism of protein. The increase in stored carbohydrate that can occur under the influence of growth hormone in suitable animals appears to be associated with depression in the rate of oxidation of carbohydrate. Under the influence

of growth hormone the rate of transport of fat to the liver may be increased. The loss of adipose tissue that treatment with growth hormone can bring about probably results from an increased rate of oxidation of fat, together with a diminished rate of synthesis of fat from carbohydrate. There has recently been demonstrated an important in vitro action of growth hormone on the formation of fat from carbohydrate in adipose tissue, which suggests that growth hormone may exert a direct action on the rate of anabolism of fatty acids (Winegrad *et al.*).

The administration of growth hormone can bring about an increase in the production of milk by cows in declining lactation, and the hormone appears also to play some part in the development of the mammary gland in the hypophysectomized rat. A stimulation of mitosis in numerous tissues, including those of the adrenal cortex, has been described in growth-hormone treated animals.

Clearly growth hormone plays an important role in the regulation of protein and fat metabolism, as well as greatly influencing the metabolism of carbohydrate.

THE DIABETOGENIC EFFECT OF GROWTH HORMONE

Between 1924 and 1932, Houssay and his colleagues first demonstrated the importance of the anterior pituitary gland in carbohydrate metabolism (1936). They found that removal of the pituitary gland or of its anterior portion greatly increased the sensitivity of an animal to the hypoglycaemic action of insulin and alleviated the severity of pancreatic diabetes. For example, glycosuria, ketonuria, and the negative nitrogen balance were all reduced by removal of the pituitary gland from a depancreatised animal. As a corollary to their observations, Houssay and his colleagues showed that if anterior pituitary preparations were administered to hypophysectomized, depancreatised animals the symptoms of diabetes reappeared or were intensified. Furthermore, the administration of suitable anterior pituitary extracts to the partially depancreatised dog in which sufficient pancreas remained to prevent the appearance of diabetes could induce the appearance of an intense diabetes (Houssay *et al.*, 1932). Quite independently H. M. Evans and his colleagues observed the appearance of diabetes in normal dogs which had been treated for a long time with growth-promoting extracts of ox anterior pituitary gland. Later Young (1937) showed that a short period of treatment of the intact adult dog with growth-promoting ox anterior pituitary extract could bring about a diabetes that persisted indefinitely after cessation of the injection of the pituitary extract.

The idea that the growth hormone of the anterior pituitary gland might not only stimulate growth but also be diabetes-inducing under some conditions emerged from these earlier investigations, and in 1949 Cotes, Reid, and Young showed that treatment with purified ox pituitary growth hormone could induce a condition of diabetes mellitus in normal adult cats. Subsequently this observation has been confirmed by other workers using intact or partially depancreatised dogs or cats (Houssay and Anderson, 1949; Campbell, Davidson, and Lei). Raben and Westermeyer (1952), however, claim to have obtained from pig pituitary tissue a preparation of growth hormone devoid of a diabetes-inducing effect in dogs. Nevertheless, other investigators have been able to demonstrate a diabetes-inducing action of the Raben and Westermeyer preparation of growth hormone. Recently Luft and his colleagues (Luft *et al.*, 1958; Ikkos *et al.*, 1958), have found that the administration of human pituitary growth hormone to hypophysectomized human patients with controlled diabetes leads to a striking exacerbation of the diabetic condition, including an intense ketonuria.

Although in some species under suitable conditions the administration of growth hormone alone is sufficient to induce a diabetic state, in other species the administration of growth hormone with corticotropin or with adrenal steroids can also induce diabetes. For instance, the administration of growth hormone, together with adrenal steroids or corticotropin, can bring about a diabetic condition in the intact rat, although the administration of growth hormone alone does not induce diabetes in this species. The secretions of the thyroid gland and of thyrotropin may also act synergistically with growth hormone in the induction of diabetes under the influence of crude anterior pituitary extracts. Nevertheless, the balance of evidence suggests that growth hormone was the principal diabetogenic agent in the extracts employed by Houssay and his collaborators in their investigations that first demonstrated the diabetes-inducing influence of pituitary preparations. It may be noted that the diabetes produced by growth hormone and that produced by corticotropin may be analogous to the diabetic conditions that can complicate acromegaly and Cushing's syndrome respectively.

It is convenient to distinguish two types of diabetes that may be induced in suitable animals by the administration of crude growth-promoting extracts or of purified growth hormone. The temporary diabetes that ceases shortly after the administration of the hormone is stopped can be termed idiohypophyseal diabetes, while metahypophyseal diabetes can be used to denote the condition that persists after the treatment with anterior pituitary extract or growth hormone is stopped and may, in the dog, persist indefinitely.

Idiohypophyseal diabetes can be induced by the administration of growth-promoting pituitary extracts to adult dogs, cats, ferrets, monkeys, rabbits, goats, and probably some other species. The administration of crude extracts of ox pituitary gland, or of large doses of purified growth hormone, does not induce diabetes in the intact rat. Although the adult dog and cat respond to the administration of growth promoting ox pituitary extracts with the exhibition of frank diabetes, this is not true when large doses of a similar extract are given to kittens or to puppies (Young, 1941). Young has found that idiohypophyseal diabetes is not induced in pregnant or lactating cats (Young, 1946). It can be said, therefore, that the effect has so far been elicited in adult nonpregnant, nonlactating members of a limited number of species, but it must be emphasized that the effects of appropriate doses of growth hormone have so far been investigated in only a limited number of species of animal.

In the intact adult dog or cat the administration of 1 to 5 mg./kg./day of purified ox growth may induce a diabetic condition. The administration of the hormone is accompanied by a gain in body weight and nitrogen retention and the blood sugar does not rise significantly above normal until 2 to 5 days after the daily administration of growth hormone has begun. The rise in blood sugar level may be associated with lipaemia, ketonaemia, polyuria, polydypsia, glycosuria, and ketonuria, and in dogs diabetic coma and death may result from the administration of growth hormone.

As the result of the administration of the growth hormone the β-cells of the islets of Langerhans of the pancreas undergo characteristic changes, the first of which is diminished staining power of the cytoplasmic granules (Richardson and Young, 1938; Richardson, 1940). Subsequently extensive and complete degranulation may occur and hydropic changes set in. The development of these histological changes is associated with a diminution in the amount of biologically active insulin extractable from the pancreas.

If the diabetic condition persists when treatment with growth hormone is stopped, a metahypophyseal diabetes may be said to exist. The ketonaemia and ketonuria may disappear and the lipaemia subside, but hyperglycaemia, glycosuria and polyuria persist. Glycosuria may be very substantial in metahypophyseal diabetic dogs but they can usually live without treatment with insulin, although the administration of insulin is associated with a marked improvement in vigour and appetite, and a rise in body weight. Even though the metahypophyseal diabetic dog can usually survive without treatment with insulin, when insulin is administered in amounts sufficient to control glycosuria and hyper-

glycaemia, the amount of insulin needed is often greater than that required by comparable depancreatised dogs (Marks and Young). Removal of the pancreas of a metahypophyseal diabetic dog may result in a fall in insulin requirement (Marks and Young, *loc. cit.*).

In dogs with metahypophyseal diabetes, the islets of Langerhans may be few in number and those that persist are smaller than normal. The β-cells may be degranulated and atrophic, and the ratio of β-cells to α-cells is much below normal. Extensive hyalinization of the islets may also be seen (Richardson and Young, 1938; Richardson, 1940). The amount of insulin that can be extracted from such a pancreas is very small.

In the cat, on the other hand, metahypophyseal diabetes may not persist indefinitely and if the animal is allowed to remain untreated for many months the condition may undergo spontaneous remission (Young, 1948). In the cat the most striking histological change in the islets of Langerhans of the pancreas is extensive hydropsis, and this hydropsis may persist even though the diabetes shows complete remission. The hydropic state of the islets is associated with a subnormal pancreatic insulin content (Young, 1958).

IS GROWTH HORMONE ITSELF DIABETES-INDUCING?

As has been stated above, the claims of Raben and Westermeyer to have prepared growth hormone not diabetes-inducing in suitable animals have not been supported by other investigators. The fact that the administration of growth hormone to suitable animals consistently induces diabetes might be interpreted as indicating the presence of a diabetogenic impurity in all the preparations of growth hormone so far prepared. If this were so, then it should be possible to dissociate the diabetogenic and growth-promoting activities of growth hormone, but so far this has not been possible. Attempts have been made to distinguish between the two activities of growth hormone by examining the ratio of growth-promoting activity to diabetes-inducing activity in preparations of growth hormone obtained by different methods from similar materials, and also of growth hormone subjected to different partially inactivating or inactivating procedures. If the growth-promoting and diabetes-inducing activities were associated with different substances, or even with different portions of the same molecule, one might expect to find conditions in which the loss of activity of one type would be greater than that of another (Reid; Young, 1953).

Since human growth hormone is very different chemically from ox growth hormone (Li, 1957), having a molecular weight of about half

that of the ox hormone and quite a different isoelectric point, the fact that the human material possessed a striking diabetes-intensifying effect in the human being (Luft *et al.*; Ikkos *et al.*) is most striking, and supports the view that the growth-promoting and diabetes-inducing actions of the hormone are not separable.

Young (1939, 1941, 1945, 1951, 1953) has supported the view that the diabetes-inducing action of growth hormone is compatible with the general physiologic significance of this hormone. He has pointed out that growth hormone is not diabetogenic in all species of animal, but in those in which it is active in this way the effectiveness in inducing diabetes is seen only in adult animals not pregnant or lactating. When growth hormone is administered to growing puppies or kittens, for instance, the stimulation of growth is not associated with the induction of diabetes. The action of growth hormone in stimulating the milk secretion of cows in declining lactation may be related to the lack of diabetogenic activity of growth hormone in lactating animals.

Young has argued that only those animals which cannot respond to the administration of growth hormone by extra growth, increased secretion of milk, and possibly of additional foetal growth are liable to exhibit diabetes as the result of the action of exogenous growth hormone. Whether or not diabetes actually develops as the result of the injection of growth hormone into a suitable animal depends on whether the food intake of the treated animal is maintained. The diabetes-inducing action of growth hormone is not seen in starving animals, although, if food intake is kept at the preinjection level, the daily administration of growth hormone readily induces a diabetic condition in suitable experimental animals. Since the injection of growth hormone often induces polyphagia, the consumption of extra food probably exacerbates the diabetes-inducing effect of the hormone particularly so in the absence of a means by which the protein and carbohydrate of the food can be physiologically utilized in an anabolic process such as growth, or even milk production.

Young has emphasized the importance of the availability of sufficient insulin from the pancreas in the nature of the response of the normal animal to the administration of growth hormone. But species differences cannot be neglected in this respect. Despite these species differences it can be said that the growth-promoting action of growth hormone depends on the availability of insulin in the body, and that the diabetes-inducing action of the hormone may be seen when the available insulin is insufficient in amount to prevent its development. An important element in the growth-stimulating action of growth hormone, at least in some species, may be the action of the hormone in rendering extra in-

sulin available either by stimulation of insulin secretion by the pancreas or by induction of its release from a bound form in blood or tissues. In species in which growth-hormone can induce an excessive secretion of insulin by the pancreas diabetes and islet degeneration may result.

GROWTH HORMONE AND THE SECRETION OF INSULIN BY THE PANCREAS

Randle (1955) and Randle and Young (1956), using the stimulation of glucose uptake by isolated rat diaphragm as a measure of insulin activity, have found high activity in blood plasma in human acromegaly and pituitary gigantism, and also in cats treated with growth hormone. On the other hand, no rise was found in normal rats treated with growth hormone. Blood plasma insulin activity was found to be low in human hypopituitarism and in hypophysectomised rats, and the low level in the blood plasma of the hypophysectomised rat was restored to normal by treatment of the animals with growth hormone.

Bennett has used the technique for assessing insulin secretion, devised by Houssay, which involves transplantation of the pancreas of experimentally treated donor dogs into the circulation of recipient depancreatized dogs, and measurement of the change of blood sugar level in the recipient animals. These experiments showed that acute treatment of the donor dogs with growth hormone stimulates the secretion of insulin by the pancreas.

Indirect evidence that growth hormone can stimulate the secretion of insulin by the pancreas of the cat was provided by the experiments of Milman, de Moor, and Lukens, and Lukens and McCann. They found that growth hormone fails to induce nitrogen retention in the hypophysectomised-depancreatised cat. When depancreatised cats, maintained on a constant dosage of insulin, received a single injection of growth hormone, the nitrogen storage induced was about half that seen in normal cats given the same amount of growth hormone. But if, at the same time as the growth hormone was injected, the amount of insulin was greatly increased, the amount of nitrogen retained was similar to that observed in normal animals. Variation in the insulin dosage alone did not alter the nitrogen excretion. Lukens and his colleagues conclude that insulin is essential for the protein anabolic effect of growth hormone, and that an increased secretion of insulin presumably occurs in response to the administration of growth hormone in the normal cat. In the human being Conn and Louis have reported that a crude pituitary extract, which certainly contained growth hormone according to its method of preparation and which was diabetes-inducing in dogs,

elicited a fall of blood sugar level when administered to a patient with hyperinsulinism. Thus the same extract was apparently capable of stimulating insulin secretion in a patient with hyperinsulinism and of inducing diabetes in a normal dog.

As has been pointed out above, treatment of the intact rat with growth hormone led to no rise of blood-plasma insulin activity in the experiments of Randle and Young (1956). Evidence against the view that treatment of the rat with growth hormone leads to an increased secretion of insulin by the pancreas had previously been provided by Anderson and Long and by Scott and Engel.

We can conclude that in some species, possibly those in which growth hormone is diabetogenic, growth hormone probably induces a significant increase in the rate of secretion of insulin by the pancreas, whereas in others, notably the rat, evidence for such an effect is lacking.

The mechanism whereby growth hormone induces such a secretion of insulin is not clear. But since repeated injections of glucose can bring about degenerative islet changes in the cat, for example, (Dohan and Lukens) it is clear that the pancreatic islets may degenerate as the result of excessive physiologic stimulation. The degenerative changes in the islets seen in diabetes induced by treatment with growth hormone would therefore accord with the view that, by direct or indirect action, growth hormone can stimulate the islets of Langerhans of the pancreas to secrete extra insulin, in some species at least.

THE INSULIN-LIKE ACTION OF GROWTH HORMONE

As has been pointed out above, the diabetes induced in suitable animals by the daily injection of growth hormone does not appear for some days after the treatment with hormone has begun, and is then dependent upon an adequate food intake. The immediate response to a single injection of growth hormone is, in many instances, a fall of blood sugar level. This is seen particularly in fasting animals, whether normal or hypophysectomised. Since an effect of this sort can be elicited in acutely depancreatised or eviscerated animals, it is not due simply to the secretion of insulin by the pancreas under the influence of growth hormone. Some days after removal of the pancreas the injection of growth hormone no longer brings about a fall of blood sugar, but may then induce a substantial rise. These observations suggest that some insulin must be present in the tissues in order that the hypoglycaemic action of growth hormone be manifest. Ottaway has suggested that growth hormone may release insulin from a bound form in nonpancreatic tissues, and the fact that the full picture of diabetes does not develop for

some days after the cessation of insulin therapy of depancreatized animals (cf. Spiro, Ashmore, and Hastings) agrees with the view that in acute pancreatic diabetes some insulin remains in the tissues for at least some days, possibly in a bound form from which it can be released by growth hormone.

The in vitro addition of growth hormone to isolated diaphragm from hypophysectomised rats (and according to Ottaway also with diaphragm from intact rats) results in a stimulation of glucose uptake by the diaphragm. This may be a direct insulin-like action of growth hormone or it may result from a release of bound insulin under the influence of growth hormone (Ottaway). Although no certain conclusion can be drawn it is likely that the insulin-like action of growth hormone does depend on the availability of insulin in the tissues, and growth hormone itself alone may possess no insulin-like action.

GROWTH HORMONE AND THE SECRETION OF GLUCAGON

The administration of growth hormone to an animal may induce histologic signs of stimulation of the α-cells of the pancreatic islets (Mosca, Ferner). At the same time there is evidence of the liberation into the pancreatic blood stream of a hyperglycaemic substance. Since, moreover, radioactively labelled growth hormone is localized to a significant extent in the pancreatic islets, probably in the α-cells, it is tempting to speculate that a significant action of growth hormone on the pancreas may be to induce the secretion of glucagon. However, under the influence of growth hormone serotonin may be liberated into the portal blood stream of a completely depancreatized animal (Sirek and Best) and since serotonin can induce a rise of blood sugar no safe conclusion can yet be made that growth hormone stimulates the secretion of glucagon although this may well be so.

GROWTH HORMONE AND INSULIN IN GROWTH AND DIABETES

In animals with real or potential deficiency of insulin, growth hormone may exert a diabetes-inducing or diabetes-intensifying action that can obscure or replace its normal growth-promoting, nitrogen-retaining action. But, under suitable conditions, the simultaneous administration of growth hormone and insulin can restore the growth-promoting action of the former (see Young, 1941; Gaebler and Robinson; Milman, de Moor, and Lukens). The question therefore arises as to whether or not the action of insulin alone can sometimes lead to nitrogen retention and true growth. Best and his colleagues (Salter *et al.*) have shown that the

administration of insulin alone to hypophysectomised rats can induce a substantial increase in body weight. Scow believes that such a stimulation of growth depends on the increase in food intake brought about by the treatment with insulin. Nevertheless, there is little doubt that in suitable circumstances treatment with insulin alone can induce some nitrogen retention in the intact animal. In agreement with this Manchester and Young (1958a,b) find that insulin in vitro can stimulate the incorporation of radioactive amino acids into the protein of isolated rat diaphragm and that the addition of glucose to the medium in which the tissue is suspended makes little difference in the result obtained in the absence of glucose. The only exception to this was with alanine with which, as had previously been found by Sinex, MacMullen, and Hastings, the addition of glucose to the medium depressed the apparent incorporation into protein of the amino acid, in the presence or absence of insulin. Manchester and Young (1958a,b) bring forward evidence that this apparent depression of the incorporation of alanine into protein by the addition of glucose to the medium is factitious, and due to dilution of the radioactive alanine by transamination with pyruvate formed from the glucose.

It seems, therefore, that in vitro as well as in vivo insulin is capable of stimulating protein biosynthesis and that this stimulation is not obviously dependent upon the action of insulin in promoting carbohydrate uptake.

Since Young (1941) and Gaebler and Robinson with the dog and Milman, de Moor, and Lukens with the cat find that the nitrogen-retaining action of growth hormone depends on the availability of extra exogenous insulin when endogenous insulin is actually or potentially deficient, it is tempting to suggest that in all circumstances the growth-promoting action of growth hormone in the normal animal depends on the secretion of additional insulin by the pancreas when growth hormone is administered, the diabetes-inducing action of the growth hormone becoming manifest when sufficient extra insulin is not available (cf. Young, 1953). But undoubtedly important species differences exist in this respect since Scow has found that growth hormone alone can stimulate growth and nitrogen retention in the hypophysectomised-depancreatised rat maintained on a steady food intake and with a constant dosage of insulin. As Young (1953) has pointed out, the rat differs in some important respects from many other species of animal in that it continues to grow slowly throughout its life, without closure of the epiphysis. This may be related to the fact that the administration of growth hormone alone does not induce diabetes in the intact rat (Young, 1953). Nevertheless, the presence of some insulin appears to

be necessary for the growth-promoting action of growth hormone in the rat, and there can be little doubt that the action of insulin is of importance in permitting the growth-promoting action of growth hormone to become manifest. It is probable that growth hormone can also stimulate protein synthesis by a mechanism other than that involving dependence on insulin secretion though the nature of such a mechanism is at present obscure. The choice of substrate to provide energy for metabolic purposes may be of particular importance in this connexion since extra fat is catabolised as the result of the administration of growth hormone, while under the influence of insulin fat storage is promoted.

ANTAGONISM BETWEEN GROWTH HORMONE AND INSULIN WITH RESPECT TO CARBOHYDRATE METABOLISM

In a sense a paradox exists in the fact that growth hormone is believed to promote the secretion of insulin by the pancreas in some species, and to depend on the availability of insulin in order to exert its normal nitrogen-retaining action, while at the same time growth hormone is concerned with a depression of carbohydrate utilisation in muscular tissues and with an action antagonistic to that of insulin in promoting carbohydrate utilisation in muscle. But the experimental facts are clear. The administration of growth hormone can depress or abolish the hypersensitivity of the hypophysectomised animal to the hypoglycaemic action of insulin, and can also greatly depress the hypoglycaemic action of insulin in normal animals.

This action can be seen in the absence of the adrenal glands. The injection of growth hormone into hypophysectomised rats can prevent the abnormal fall of muscle glycogen content which occurs during fasting in the absence of the pituitary gland. This effect of growth hormone has been called its myoglycostatic action (Russell), and an action of this sort can also be elicited when growth hormone is administered to fasting normal animals.

When growth hormone is injected into a normal or a hypophysectomised fasting rat the in vitro glucose uptake of diaphragm excised from the animal 3 to 24 hours later is much depressed, and the isolated diaphragm is much less responsive to the action of insulin in vitro than is diaphragm from untreated control animals. In hypophysectomised-adrenalectomised rats growth hormone does not produce as big a depression of glucose uptake by the diaphragm in vitro as it does in the hypophysectomised rat. Nevertheless, as big a depression can be induced when the hypophysectomised-adrenalectomised rat is

given both growth hormone and adrenal cortical extract. The latter, by itself, induces no depression. Park, Krahl, and their colleagues conclude from these observations that the inhibitory action of injected growth hormone upon the uptake of glucose by diaphragm in vitro depends on an in vivo transformation of growth hormone to an inhibitory substance, the transformation involving adrenal hormones. Subsequently Bornstein and Park (1953) showed that the uptake of glucose in vitro by diaphragm from normal fasting rats was lower in the presence of serum from alloxan-diabetic rats than it was in the presence of serum from normal rats. Serum from alloxan-diabetic rats which had been either adrenalectomised or hypophysectomised did not contain the inhibitory substance. The injection of growth hormone or of cortisone separately into alloxan-diabetic hypophysectomised rats did not induce the reappearance in the serum of the inhibitory substance, but the injection of both growth hormone and cortisone simultaneously did so. The in vitro addition of growth hormone and cortisone to the serum had no effect on the glucose uptake of the isolated diaphragm of the normal fasting rat. Bornstein (1953) has found the inhibitory substance to be present in the lipoprotein fraction of the serum of alloxan-diabetic rats. The inference to be drawn from these experiments of Bornstein and Park, and of Bornstein is that the active lipoprotein is formed in the rat only in the presence of growth hormone and adrenal steroids, and may represent the previously postulated transformation product of growth hormone.

In somewhat similar experiments with cats, in which the inhibitory properties of the serum were measured in terms of the neutralization of the action of insulin on isolated rat diaphragm, Vallance-Owen and Lukens conclude that their inhibitory factor from cat plasma is not a lipoprotein but is found in the γ-globulin fraction of plasma proteins. Unfortunately, in their experiments Vallance-Owen and Lukens did not show that the pituitary factor involved was growth hormone although this was very likely.

Other investigations also point to an antagonism between insulin and growth hormone, or a substance produced under the influence of growth hormone, with respect to the uptake of glucose by muscle tissue. Two questions then arise. What is the mechanism of this antagonism, and what is its physiological significance?

Cori and his colleagues have pointed to the enzyme hexokinase as a probable seat of antagonism between pituitary hormones and insulin but, in the writers' view, the evidence in support of this idea is not conclusive. The earlier demonstration of an in vitro antagonism between pituitary extracts and adrenal steroids on the one hand, and insulin on the other, has been difficult to reproduce, though Ilyin and

species under particular conditions, may represent a pathologic outcome of the excessive emphasis of physiologic processes.

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Chapter 18

OTHER HORMONES

Bernardo A. Houssay

HYPOPHYSIS

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3. After total hypophysectomy or removal of the *pars distalis* the animals become extremely sensitive to the hypoglycemic and toxic action of insulin (Houssay and Magenta, 1924, 1927, 1929), much more than the adrenalectomized animals (de Bodo and Sinkoff, 1953). Small doses of insulin, which have no effect in normal controls, provoke intense hypoglycemia, convulsions, and death. The animals that recover do not regain a normal sugar level for several hours. There is also increased sensitiveness to other hypoglycemic agents such as phloridzin (Houssay and Biasotti, 1930b); there is also marked secondary hypoglycemia following glucose or adrenalin hyperglycemia (Braier, 1931; de Bodo and Sinkoff, 1953). This sensitivity to insulin is also found in human pituitary insufficiency and the injection of insulin is dangerous in these cases. Lack of hypophyseal growth hormone and adrenal hypofunction contribute to establish the hypersensitiveness. Treatment with somatotropin or glucocorticoids can correct the hypersensitiveness and even produce resistance to insulin in normal or hypophysectomized dogs (de Bodo and Sinkoff, 1953).

4. Hyperglycemia provoked by adrenalin and other agents is less marked than in normal animals (see literature in: Houssay, 1942);

5. Extirpation of the hypophysis or its anterior lobe (*pars distalis*) is followed by marked attenuation of pancreatic and phlorhizin diabetes (Houssay and Biasotti, 1930). The decrease in severity of pancreatic diabetes caused by hypophysectomy (Houssay and Biasotti, 1930) is shown by: (1) lower hyperglycemia and less glycosuria; in fasting animals hypoglycemia may develop, which can be fatal if not promptly treated with glucose; (2) marked sensitiveness to insulin; (3) the capacity of utilizing endogenous and exogenous glucose, though below normal, is greater than that of diabetic animals, (4) protein and fat catabolism are lower than in pancreatectomized animals, therefore, there is less ketonuria and urinary nitrogen elimination; (5) the urinary D/N ratio is low; and (6) loss of weight is less marked and survival is prolonged.

In man, diabetes can also be alleviated by hypophysectomy or pituitary insufficiency. This "Houssay syndrome" has been observed in 17 cases (Calvert and Caplin).

Metabolic disturbances of tissues removed from pancreatectomized-hypophysectomized animals and observed in vitro are less marked than in those of pancreatectomized animals and even may not be present. This has been demonstrated for glucose consumption of muscle and heart of dogs and for deamination and ketogenesis (Stadie *et al*) and lipogenesis from acetate (Brady *et al*) by the liver of cats.

Fasting hypoglycemia in hypophysectomized animals and the attenua-

tion of pancreatic diabetes following hypophysectomy are not due to predominance of insulin, because they take place in animals without the pancreas (Houssay and Biasotti, 1930a). Using minute amounts of C^{14} glucose, hypophysectomized dogs were shown to have a smaller glucose pool and lower rates of glucose inflow from the liver to blood and of glucose outflow from the plasma to the cells than in normal dogs (Steele, Wall, de Bodo and Altszuler). The sensitivity to insulin has two causes: (1) the consumption of sugar is increased in tissues of hypophysectomized dogs; (2) during hypoglycemia they have no increase in glucose inflow from the liver to blood, as the normal dogs have. All these disturbances can be corrected by somatotropin (de Bodo and Altzuler).

The peripheral consumption of glucose is slower in dogs and increased in rats and rabbits (Houssay, 1942; de Bodo and Altzuler).

The proper administration of extracts or hormones of *pars distalis* corrects all the disturbances resulting from hypophyseal insufficiency; larger doses provoke disturbances in the opposite direction. There is no longer hypoglycemia in fasting, hypersensitiveness to phlorhizin, and secondary hypoglycemia following hyperglycemia owing to glucose or adrenalin. There is marked resistance to insulin. Hyperglycemia caused by adrenalin and other substances is increased. Glycogen stores, specially those of muscles, are not depleted by fasting; this glycomyostatic action is observed in absence of adrenals.

Hypophyseal Diabetogenic Activity

The diabetogenic activity of the hypophysis was discovered by Houssay and Biasotti, in 1930, who observed: (1) severity of pancreatic diabetes decreases following extirpation of the hypophysis or its *pars distalis* (in toads and dogs); and, (2) implantation or injection of *pars distalis* re-establishes the intensity of pancreatic diabetes attenuated by hypophysectomy or even increases it (in toads). These constituted the first demonstration that: (1) pancreatic diabetes is due not only to lack of insulin, but also to the presence of hypophyseal hormones, which increase the severity of diabetes; (2) pituitary hormones have a continuous physiological action on the carbohydrate metabolism in the normal and diabetic state; and, (3) carbohydrate metabolism is regulated by a balance of hormones (pancreatic, hypophyseal, adrenal, etc.) which are in part antagonistic and in part synergic; (4) in diabetes, there is a disturbance in the balance of endocrine regulatory factors.

The diabetogenic activity of pituitary gland and its hormones has been demonstrated in: (1) hypophysectomized-pancreatectomized animals (Houssay and Biasotti, 1930, Houssay, Biasotti, di Benedetto and

There are antagonistic and synergic effects between the hypophysis and the pancreas: (1) there is marked hypersensitiveness to insulin in hypophysectomized animals, and treatment with hypophyseal extracts or hormones causes insulin resistance; and (2) reduction of the pancreatic mass facilitates the production of diabetes by treatment with hypophyseal hormones or extracts, doses one one-hundredth to one five-hundredth of that necessary to provoke diabetes in a normal animal can be *efficacious after partial pancreatectomy.*

Previous treatment with hypophyseal extract or hormones at first facilitates and later antagonizes the effect of insulin on the uptake of glucose and glycogen formation of the isolated diaphragm (Krahl, 1956).

The growth hormone and insulin act synergically in the fixation of nitrogen and growth. Somatotropin alone does not have anabolic protein action and is unable to produce growth in totally pancreatectomized rats, cats and dogs.

Role of the Adrenals

The adrenals exert multiple influences on hypophyseal diabetes: (1) adrenocorticotropin can have a diabetogenic effect by provoking corticoadrenal hypersecretion; (2) corticoadrenal hormones are either necessary for or reinforce the diabetogenic activity of hypophyseal extract or of somatotropin; (3) summation of effects of hypophyseal and adrenal hormones has been observed; and (4) in some cases, corticoids balance or regulate the action of the hypophysis (de Bodo and Sinkoff, 1953b).

The presence of the adrenals or of corticoadrenal hormones facilitates considerably the diabetogenic action of the hypophysis in cats, dogs, rats and toads. This action has not been observed in adrenalectomized-pancreatectomized cats (Long and Lukens, 1936), but it has been obtained in adrenalectomized dogs with ample pancreatectomy, maintained alive with desoxycorticosterone or with sodium chloride alone. Diabetes has also been obtained by hypophyseal treatment in toads after removal of the hypophysis, pancreas, and adrenals. Anterohypophyseal extract increases muscle glycogen content in adrenalectomized rats. Somatotropin restores to normal insulin resistance that has been lowered by adrenalectomy in the dog. Somatotropin also increases insulin resistance in adrenalectomized-hypophysectomized dogs, and a diabetic glucose tolerance curve is observed (de Bodo and Sinkoff, 1953b). Somatotropin and prolactin produce diabetes in adrenalectomized, hypophysectomized *dogs with pancreas surgically reduced* (Houssay and Penhos).

Summation of effects of adrenal and hypophyseal extracts has been

observed in the following cases: (1) glycosuria in adrenalectomized-pancreatectomized animals; (2) muscle glycogen storage; (3) diabetogenic activity of corticotropin and somatotropin; (4) diabetogenic effect of cortisone and somatotropin; and (5) inhibition of glucose uptake by the isolated diaphragm.

According to de Bodo and his associates, an equilibrium between somatotropin and corticoids regulates normal carbohydrate metabolism. Somatotropin, given to hypophysectomized or hypophysoadrenalectomized dogs, rapidly provokes insulin resistance, diabetes, and later, toxic symptoms. If, however, cortisol is given previously or simultaneously, carbohydrate metabolism returns to normal and neither diabetes nor toxic symptoms appear (de Bodo and Sinkoff, 1953).

Hypophysis and Human Diabetes

Hypophyseal hyperfunction is the probable cause of the high frequency of diabetes of glycosuria in acromegaly. Coggeshall and Root (1940) mentioned 36 per cent glycosuria and 18.9 per cent diabetes in 153 cases of acromegaly. In the survey made by Atkinson, glycosuria was found in 268 out of 817 cases published (32.8 per cent). Probably, hypophyseal hyperfunction also exists in cases of diabetes beginning during a period of rapid growth. There is no proof that human diabetes is due to hypophyseal hyperfunction; but hypophyseal function increases the severity of the symptoms of human diabetes. Hypophysectomy diminishes the intensity of diabetes and has been tried in cases of malignant diabetes (Luft *et al.*; Kinsell, 1957). Corticotropin or human somatotropin injections increase the severity of human diabetes in the majority of cases. In some cases of diabetes in acromegalics there is insulin resistance, but not in others; probably, in the former, hypophyseal hormones (somatotropin or corticotropin) are active, just as in hypophyseal diabetes; on the other hand, insulin resistance would be normal in cases comparable to experimental metahypophyseal diabetes.

ADRENALS

The adrenal medulla can be extirpated without causing changes in the blood sugar level, glycogen content, or the blood sugar tolerance curves. The course and severity of pancreatic (Houssay and Lewis), phlorhizin or hypophyseal (Houssay *et al.*, 1933) diabetes is not altered. Adrenalin, secreted by the adrenal medulla, provoked by several agents (hypoglycemia, etc.) can provoke a transitory increase in blood sugar, and accelerates recovery from hypoglycemia, e.g., insulin hypoglycemia. Diabetes has been observed in some cases of human pheochromocytoma

and has been alleviated or has disappeared in a few observations, after resection of the adrenal tumor.

The adrenal cortex plays an important part in carbohydrate metabolism and in diabetes.

Adrenal Insufficiency

Disturbances in carbohydrate metabolism observed in adrenalectomized animals are similar to those of hypophysectomized animals, but they are usually less marked. In well-fed animals, carbohydrate levels and utilization are almost normal, but blood sugar and glycogen content are low in the postabsorptive state, and decrease in fasting and conditions of stress. At first hepatic glycogen and later the blood sugar falls, but muscle glycogen falls only in the advanced stages when circulation fails. In the adrenalectomized dog, the recovery of muscle glycogen after fatigue is retarded and it is not stored normally after the injection of glucose; adrenal extracts correct these disturbances, even if the pancreas has been removed. Adrenalectomized animals are usually more sensitive to insulin than normal ones, but less so than hypophysectomized animals; adrenal extracts and corticoids correct this hypersensitiveness. The glucose tolerance curve in the dog is usually high and prolonged or has a higher peak and greater fall. Lipogenesis is diminished in fasting and stress, and it is difficult to produce a fatty liver. Phlorhizin glycosuria is less marked. Pancreatic diabetes is less severe in the adrenalectomized rat, dog, and cat (Long and Lukens), toad (Houssay and Biasotti, 1931, 1936) and man. Comparing pancreatectomized-adrenalectomized with pancreatectomized animals; the following differences have been found: in the latter group, hyperglycemia, glycosuria, and ketonuria are less marked, and there is a low urinary D/N ratio; survival is longer.

Action of Adrenal Hormones

Adrenal extracts and the glucocorticoids of the adrenal cortex markedly increase liver glycogen, and slightly raise the blood sugar of fasting normal, adrenalectomized, or hypophysectomized animals. Muscle glycogen is not increased in fasting animals, but it does increase when they are fed carbohydrates.

Prolonged treatment with large doses of cortisone produces considerable hyperglycemia in rabbits, hyperglycemia and glycosuria in guinea pigs, and hyperglycemia in chicks.

Diabetes has been obtained by glucocorticoids in rats: (1) with pancreatectomy and hypophysectomy, (2) with partial pancreatectomy, and (3) in normal rats (Ingle). Desoxycorticosterone is less active, but

it can provoke glycosuria in partially pancreatectomized rats. Corticoids produce glycosuria, hyperglycemia, marked insulin resistance, and a high tolerance curve. Glycosuria and hepatic glycogen increase in proportion to the dose of corticoid given. Cortisone exerts its glycosuric effect very slowly and progressively, reaching a maximum in 7 to 14 days; afterward, the effect diminishes in spite of continued treatment. After 19 to 25 days of treatment, glucose tolerance diminishes and after 25 days, all the animals show hyperglycemia.

Corticotropin releases corticoadrenal hormones, thus producing a transitory diabetogenic effect (glycosuria and hyperglycemia) in normal rats.

Intense treatment with larger doses of cortisone produces only slight hyperglycemia in normal cats and dogs.

When the pancreas was surgically reduced to 16 to 23 per cent (average 20 per cent) of its initial weight, a transitory corticoid diabetes or a persistent metacorticoid diabetes can be produced by cortisone, hydrocortisone, or some other synthetic with corticoid action (Houssay *et al.*, 1954). Corticotropin administered to hypophysectomized dogs suppresses sensitiveness to insulin, secondary hypoglycemia after glucose, and the glycemic response to adrenalin. Higher doses produce hypercorticalism: resistance to insulin, high tolerance curve, supernormal response to adrenalin.

Cortisol has a more potent effect than cortisone in producing transitory diabetes in the rat, permanent diabetes in the dog, and in diminishing glucose tolerance in diabetic patients with Addison's disease. In normal human subjects and in nondiabetic patients, prolonged administration of cortisone or corticotropin in some cases produces a slight and variable increase in the fasting blood sugar, but there is no permanent increase above the normal level. A few exceptional cases show a decrease in glucose tolerance, which returns to normal when cortisone or corticotropin is discontinued. The diabetogenic action of corticotropin, first observed by Browne was later seen in some normal human subjects (Conn). There was moderate hyperglycemia, a certain resistance to insulin, and a decrease in the re-absorption of glucose by the renal tubes, because in some cases there is glycosuria with little or no hyperglycemia. Apart from these cases, a diabetogenic action of corticotropin or corticoids has seldom been reported in the large number of papers on treatment with this hormone.

Action on the Islets

Considerable hyperplasia of the islets of Langerhans is produced by prolonged administration of cortisone in guinea pigs, normal rats, and

hypophysectomized rats and by cortisone and cortisol in dogs. The β-cells of the islets show: (1) in rats, hypertrophy and hyperplasia, but no degeneration; (2) in guinea pigs, hypertrophy and hyperplasia; some β-cells have reversible degranulation and irreversible vacuolation; (3) in rabbits and in metacorticoid diabetes in dogs, hydropic degeneration (glycogen infiltration) of β-cells and the epithelial cells of the ducts has been observed; and, (4) in dogs without diabetes, hypertrophy and hyperplasia.

Corticotropin provokes a greater mitotic activity in the β-cells, an increase in the size and number of the islets, and enlargement and degranulation of the β-cells in the rat. The increase in islet tissue is even more evident in the hypophysectomized rat.

With daily injections of different glucocorticoids (compounds A, E, F) during six months, prevention of diabetes was observed in a large proportion of subtotally pancreatectomized rats. There was an initial increase in the incidence of diabetes, and after six months the percentage of diabetic rats (Houssay, Foglia, and Rodríguez) was considerably less than in the untreated controls. All protected rats showed hypertrophy and increase in the number of islets of Langerhans, the β-cells of which had normal granulation.

Mechanism of Corticoid Action

The principal factor in the diabetogenic action of corticoids is that the amount of glucose formed by gluconeogenesis from sources other than absorbed glucose is, in cortisone diabetes, approximately seven times the amount formed in normal rats. This gluconeogenesis does not take place solely at the expense of protein. Glycosuria, observed in unanesthetized rats treated by cortisone, is due not only to the greater production of glucose, but also to impairment in the utilization of glucose; in anesthetized rats this impairment is not evident. In cortisone diabetes, there is marked resistance to insulin and a peripheral antagonism between insulin and cortisone. Excess of corticoids increases protein catabolism. Cortisone hyperglycemia has been observed in adrenalectomized and in thyroidectomized rats; it is more marked in hypophysectomized rats. Therefore, the adrenals, thyroid, and hypophysis are not necessary for the diabetogenic effect of cortisone.

The action of cortisone may be diphasic. In the rat, treatment causes a maximal glycosuria in 6 to 7 days: later, glycosuria diminishes. In the subtotally pancreatectomized rat, large doses of active corticosteroids at first increase the incidence of diabetes but later, in a great number of cases, there is a smaller incidence and marked protection.

In human subjects treated with cortisone over a long period, hypo-

glycemia and sensitivity to insulin may occur when treatment is discontinued. In 18 cases treated with cortisone, there was at first depression in glucose consumption; later, a higher rate of glucose consumption developed.

Cortisone almost always increases the severity of diabetes in the human subject, but it improved the diabetic condition in three hirsute diabetic women. In some diabetics, desoxycorticosterone diminishes resistance to insulin, probably by inhibition of the pituitary.

There are two possible mechanisms by which the favorable effect of corticosteroids may be exerted: (1) corticosteroids inhibit hypophyseal functions, especially the secretion of corticotropin. This action may take place rapidly and, after a time, it will cause atrophy and a certain degree of hypofunction of the adrenal; (2) probably, insulin secretion increases; thus, a normal blood sugar and glucose tolerance is maintained. If the effect of cortisone is intense, a transitory diabetes may be produced. In the rat there is hypertrophy and hyperplasia of the islets, and the blood sugar level returns to normal. In subtotally pancreatectomized rats, this insular hypertrophy can prevent the appearance of diabetes. If the action of cortisone is more intense and the pancreatic reserve is diminished (especially in sensitive species such as the dog, or with surgical reduction, previous damage of the pancreas, or simultaneous effects of several substances), there are marked lesions of the pancreas, hydropic degeneration (glycogen infiltration) of the β-cells, and a metacorticoid diabetes develops, i.e., a permanent diabetes, which persists after treatment has been discontinued.

Adrenals and Human Diabetes

It has not been proved that human diabetes is produced by adrenal hyperfunction; but its severity is increased by the existence of adrenal function. Adrenalectomy diminishes the severity of human diabetes and insulin requirement, and can arrest vascular lesions.

In 49 per cent of cases of Cushing's disease and of hyperplasia and tumors of the adrenal, glycosuria or changes in the glucose tolerance curve have been reported (Lukens). Subtotal extirpation of the adrenals usually improved glucose tolerance, and in some cases diabetes, which needed insulin treatment, disappeared (Sprague).

THYROID

Sugar Absorption

Thyroid hormone increases the rate of absorption from the intestine of glucose and especially of galactose, which is retarded in hypothyroid-

ism and takes place at a higher than normal rate in hyperthyroidism. This causes low blood sugar curve following oral galactose administration in hypothyroidism and a high curve in hyperthyroidism. No difference is usually present after intravenous administration (Althausen).

Blood Sugar and Tolerance Test

In hypothyroidism, blood sugar and liver glycogen are normal or subnormal, except in cases with cachexia in which they are low. Sensitiveness to insulin is increased in varying degrees in different species (Houssay, 1945, 1946).

In human hyperthyroid cases, the fasting blood sugar level is often within normal limits, although values of 120–140 mg./100 ml. may occasionally be found without a coexistent diabetes. In blood tolerance tests the initial value can be high and, after sugar administration, the increase is supernormal, but the return to normal level is observed in two hours. This higher and slightly prolonged curve has been observed in 50 per cent to 80 per cent of cases of hyperthyroidism without diabetes (literature in Houssay, 1945, 1946). In a person with hyperthyroidism, diagnosis of diabetes can be made only if the initial value is higher than 150 mg./100 ml. and the increase after sugar ingestion is to over 200 mg. (Joslin *et al.*; Wilder).

Slight spontaneous glycosuria has been found in 15 per cent to 60 per cent of cases of hyperthyroidism (Sattler; Wilder). Joslin observed glycosuria in 38 per cent of cases of primary hyperthyroidism and 28 per cent of secondary hyperthyroidism. Glucose is rapidly absorbed in relation to high metabolic rate. In moderate hyperthyroidism, sensitiveness to insulin may be diminished, but in advanced cases it may be increased (Houssay, 1945, 1946). Glycogen diminishes in hyperthyroidism, first in the liver, then in the heart and in more advanced stages, also in muscles (Houssay, 1945, 1946).

Thyroid Insufficiency and Diabetes

Striking differences are observed in accordance with the species. Surgical thyroidectomy does not modify the severity of diabetes in dogs (de Finis and Houssay; Houssay, 1945, 1946, 1948). After iodothyroidectomy, there is a slight and transitory diminution of glycemia and requirement of insulin in dogs (Houssay, Houssay and Cardeza, 1955). In rats, surgical thyroidectomy or iodothyroidectomy diminishes markedly the incidence of alloxan diabetes and prevents in large number of cases the appearance of diabetes after large (95%) pancreatectomy (Houssay, 1945; Houssay, Foglia, and Martínez, 1946). Iodothyroid-

ectomy can produce the regression of diabetes in many cases of alloxan diabetes in rats (Allegri, Bazerque and Denti).

Many antithyroid substances (*cysteine, thiouracil, etc.*) *exert an influence* on diabetes, partly by provoking hypothyroidism and partly by a direct action. The latter has been observed in thyroidectomized animals, and is connected with an increase in free SH in tissues. The following facts have been established in the rat (Martínez): (1) previous treatment with these drugs or thyroidectomy markedly increases resistance to the toxic and diabetogenic effect of alloxan; (2) they also delay the onset of diabetes after subtotal pancreatectomy in the rat and decrease its frequency; (3) propylthiouracil diminishes glycosuria of rats with total pancreatectomy, force fed, and maintained with a constant dose of insulin, and (4) propylthiouracil diminishes the sensitivity to alloxan produced by diets containing high proportion of unsaturated fatty acids.

The degradation of insulin is diminished by thyroidectomy and some sulphur compounds. The degradation is accelerated by thyroxine or triiodothyronine (Elgee and Williams, 1955).

Diabetogenic Action of Thyroid Gland

In animals with intact and healthy pancreas, thyroid administration does not produce diabetes (Houssay, 1944, 1945, 1948). Transient glycosuria has appeared in men undergoing thyroid treatment.

Daily administration of thyroid can produce diabetes in dogs only if the resistance of the pancreas has been previously diminished: (1) by resection of 80 per cent to 87 per cent of the mass of the pancreas; (2) by recent transitory alloxan or pituitary or thyroid diabetes This thyroid diabetes is transitory and disappears a few days after the administration of thyroid has ceased (Yriart, 1930; de Finis and Houssay; Houssay, 1944, 1948); but if the thyroid treatment is continued long enough the lesions of β-cells become irreversible and when the treatment is suspended, the dogs *remain* with a *permanent diabetes, metathyroid* diabetes (de Finis and Houssay, Houssay, 1944, 1946, 1948). This pancreas does not secrete more insulin (Houssay, 1944, 1945, 1946, 1948). The thyroid and metathyroid diabetes can be obtained in absence of thyroid, gonad, and adrenal medulla, but not in hypophysectomized or adrenalectomized dogs, which die as they are unable to tolerate thyroid treatment. The liver is the source of hyperglycemia in thyroid and metathyroid diabetes. Hepatectomy produces a fall of the blood sugar level until low levels and hypoglycemic symptoms occur. They can be alleviated by glucose injection.

Administration of thyroid gland or thyroxine increases the severity

of all types of experimental or human diabetes. Glycosuria, polyuria, and ketonuria increase markedly.

Thyroid treatment produces hypertrophy and hyperplasia of β-cells of the islet of Langerhans of many animal species (rats) but little or none in others (dogs). In rats, after large pancreatectomy (95 per cent), it is possible with adequate doses to observe an initial aggravation of diabetes and in a second stage, an improvement or cure of it (Martínez, 1946). The sensitivity to alloxan is increased in rats by a short previous thyroid treatment and decreased by a longer treatment (Houssay, Foglia, and Martínez).

Diabetes is not a cause of hyperthyroidism. The incidence of hyperthyroidism was 1 per cent in 32,000 cases of diabetes (Joslin *et al.*). But in hyperthyroidism there is an increase of frequency of diabetes, which was present in 2.5 per cent of cases of primary hyperthyroidism (Joslin *et al.*) and in 4.3 per cent of secondary hyperthyroidism (Joslin *et al.*) and in 5.6 per cent with nodular goiter (Wilder).

When hyperthyroidism and diabetes are simultaneously present in a subject, they exert a mutually unfavorable influence and each condition must be carefully treated. Control of hyperthyroidism (surgery, antithyroid drugs, I^{131}) decreases markedly the intensity of diabetes: life is prolonged, hyperglycemia, glycosuria, and ketonuria diminish, and insulin requirement is smaller.

SEX HORMONES

Prevention or Intensification of Diabetes

The physiologic and pharmacologic action of sex hormones on diabetes has been mainly demonstrated in white rats. After a large (95 per cent) ablation of the pancreatic mass in these animals, diabetes appears after 1 to 2 months, and its intensity increases progressively. Diabetes appears in a much higher proportion in the male than in the female (Foglia; Rodríguez). Six months after operation, 89 per cent of the males and 27 per cent of the females were diabetic.

The sexual difference is not due to different amounts of food. This was proved by experiments with paired feeding and forced feeding. The difference is maintained between males and females receiving the same amount of food.

In subtotally (95 per cent) pancreatectomized rats, removal of the testes diminishes the incidence of diabetes, and removal of the ovary increases it (Foglia, 1945).

Restitution of the ovaries by means of a graft gave considerable protection in spayed females with subtotal pancreatectomy.

The sexual difference is due to a protective action of the ovary and a provocative action of the testicle, which is shown by experiments in castration and restitution. In rats with subtotal (95 per cent) pancreatectomy, early treatment with estrogens produced a biphasic effect: first the incidence and severity of diabetes was increased in some cases, and later, diabetes was reduced or even definitely suppressed in many cases.

In force-fed rats, the appearance or intensification of glycosuria and hyperglycemia during estrogen administration has been observed: (1) in rats with partial pancreatectomy; (2) temporarily in normal rats; (3) in rats with alloxan diabetes. The diabetogenic effect of estrogens has also been observed in subtotally pancreatectomized-adrenalectomized rats maintained with subdiabetogenic doses of cortical extract and in some rats submitted to these operations, but not given cortical extracts. The intensity of the initial diabetogenic effect increases as the amount of pancreatic tissue diminishes; it is also dependent on the dose of estrogen administered, and on the diet.

The following results have been observed in totally pancreatectomized animals treated with estrogens: (1) attenuation of diabetes in dogs, monkeys, and cats; the results were striking in 40 cats, most of which died in hypoglycemia (Acevedo and Migone, 1952); (2) no effects were observed in monkeys and dogs; and (3) increase in severity of diabetes in the ferret and in rats.

The protective effect of steroids against development of diabetes following subtotal pancreatectomy in rats was first demonstrated by injecting estradiol for 6 months or estrogens or corticosteroids for several months. Androgens, on the contrary, caused an earlier appearance and increased the incidence and severity of diabetes.

In all these experiments, the incidence of diabetes was decreased by treatment with the following substances: estrone, estradiol, stilbestrol, mono-benzil-diethylstilbestrol, dienestrol, phenocycline, ethynilestradiol and ethyniltestosterone. Androgens (testosterone and methyltestosterone) markedly increased the incidence and severity of diabetes in male and female castrates. The incidence of diabetes was not modified by treatment with desoxycorticosterone. Equilenine and progesterone were inactive in the doses tested, with much higher doses diabetes was increased by progesterone and some related steroids.

Regression of alloxan diabetes was obtained by estradiol plus insulin. All the control rats remained diabetic or died during the 6 months of observation. The animals treated with insulin were in good condition, but when insulin was discontinued, all of them became diabetic, and none was cured. Estradiol benzoate produced, at the beginning, an ag-

gravation of the intensity of diabetes, and some of the animals died. Afterward a curative action was produced, and in 47 per cent of the rats, hyperglycemia and glycosuria disappeared. The injections were discontinued after 6 months, and the curative effect was maintained for 3 to 4 months (Rodriguez).

With estradiol and insulin, the curative action is more marked; it was observed in 21 out of 31 rats (65 per cent). In this type of treatment, insulin prevents the harmful effect of hyperglycemia on the β-cells of the islets of Langerhans; this allows the active agent used, in this case estradiol, to exert its curative action.

The mechanism of the initial actions of estrogens is still obscure. The diabetogenic action of diethylstilbestrol is not due to increased secretion of adrenal hormones, but the presence of cortical hormones is a conditioning factor (essential in many experiments) for this metabolic response.

Temporary diabetes has been produced in rats by estrogens, but permanent metaestrogenic diabetes has not been observed in the rats. Intolerance to estrogens and death have precluded, until now, long-term experiments in dogs. Temporary estrogen diabetes in rats was obtained only if the quantity of food was not decreased.

The preventive action of estrogens in subtotally pancreatectomized rats is not due to a difference in the amount of food ingested. With the same ingestion of food by forced feeding, the preventive action appears only in animals treated with estrogens, and not in nontreated controls. Estrogens increase the weight of the hypophysis, but reduce many hypophyseal functions. This may play a part in the mechanism of the preventive action of estrogen treatment.

The administration of estrogens in rats increased the weight of the adrenals. However, the protective action could not be attributed to the adrenals as it occurred in subtotally pancreatectomized rats, already diabetic, which were then adrenalectomized.

Action of Estrogens on the Pancreas

The protective action of estrogens seems to be due chiefly to the fact that they produce hypertrophy and hyperplasia of the islets of Langerhans and β-cells in the pancreas. The protective action is observed in the diabetes resulting from subtotal pancreatectomy, but not in that resulting from total or almost total pancreatectomy.

Hypertrophy and hyperplasia of the islets have been observed in hypophysectomized rats with subtotal pancreatectomy after estrogen treatment.

The insulin content of the pancreas is similar in rats of both sexes

and is not changed by castration, but there is more insular tissue in the pancreas of female guinea pigs and rats.

Estrogen treatment increases the insulin concentration in the rat pancreas, but not in the hypophysectomized rat. An increase of insular tissue and histologic signs of hyperactivity of the β-cells have been observed.

In subtotally pancreatectomized rats with diabetes there is degranulation and vacuolization in the β-cells, their number decreases, and there is atrophy of the islets. In animals that do not become diabetic or recover after temporary diabetes, subtotal pancreatectomy is followed by compensatory hypertrophy of the islets of Langerhans, accompanied by sclerosis in other parts of the pancreas. This hypertrophy may be considerable after one year or more.

The female rat has a greater volume of islet tissue than the male. After subtotal pancreatectomy, the incidence of diabetes is less in females than in males, but females show hypertrophy and hyperplasia of the islets of Langerhans more often than the males. Estrogens produce a rapid and marked hypertrophy of the islets with the formation of new islets. Reviews of these findings have been published by Rodríguez (1951) and Houssay *et al.* (1953–1954). The appearance of diabetes in subtotally (95 per cent) pancreatectomized rats is prevented by treatment with estrogens or propylthiouracil. When both substances are given, their protective effects are additive. There is, however, a considerable difference between results obtained with each substance alone when treatment is discontinued after 3 months. Rats treated with estrogens have hypertrophy and hyperplasia of the islets and do not later become diabetic. Rats treated with propylthiouracil rapidly become diabetic after treatment is discontinued; the protective metabolic action ceases, and there is little or no hyperplasia of the islets. Rats given both substances lose the protective effect of thiouracil when treatment is discontinued, but retain that which has been induced by estrogen.

The hypertrophy and hyperplasia of β-cells and of islets of Langerhans may be due both to an indirect and to a direct action. A direct action was observed after intrapancreatic implantation of estradiol into normal rats, guinea pigs, and cats, which showed first hyperplasia of the centroacinar cells, then newly formed islets containing hypertrophied β-cells. The formation of new islet tissue was more pronounced in parts near the estrogen implant.

It is very important to emphasize that the protective action of estrogens is produced only after rather prolonged treatment. The effect is clear after 3 months, and more marked after 6 months. The factors that

stimulate hypertrophy and hyperplasia of the islets are not known. This action can be due to: (1) variations in the blood content of insulin, glucose, or other metabolic substances; (2) direct or indirect effect on the hypophysis and other endocrine glands; and (3) direct or indirect effect of estrogens on the metabolism of the tissues or islets.

Many incomplete studies, especially of acute changes, have been performed on some phases of the metabolic actions of sex hormones, but a complete study of the changes in tissue metabolism after prolonged treatment is still lacking.

Estrogens and Human Diabetes

In the human species, diabetes is more common in women than in men, with an increase from the fourth decade of life onwards. Estrogen treatment of human diabetes has given contradictory results. Improvement has been observed in some cases of diabetes in menopausal women, but not in others.

In six acromegalic diabetics, high doses of estrogen caused marked improvement of diabetes. In one fourth of pregnant diabetic women, estrogen treatment diminished the severity of diabetes and decreased the insulin requirement.

SUMMARY

The metabolism of carbohydrates is related to that of fats and proteins. They are all governed by a balance between the different hormones that have metabolic activity.

Diabetes is a disturbance of carbohydrate metabolism in which the normal balance of the hormonal regulating factors is altered.

The role of the peripheral tissues is incompletely known. The hormonal factors have an important role, direct or indirect, in the utilization of glucose by the tissues.

In this regulation, the liver plays the most important part, since it is the organ which produces glucose and thus governs the blood sugar under the influence of the hormone equilibrium. If the liver is absent, diabetic hyperglycemia cannot occur.

The secretion of each hormone is regulated and there is a reciprocal equilibrium between them.

The endocrine secretion of the pancreas has a fundamental role since it maintains the blood sugar at a normal level and prevents its increase, and influences the production and consumption of glucose. The secretion of insulin is governed by the level of the blood sugar and vice

versa. The central nervous system is not necessary for the secretion of insulin, but the vagus has a secondary and accessory role, causing a more rapid and perfect correction of changes in the blood sugar.

The presence of the anterior pituitary hormone prevents hypoglycemia and the decrease of glycogen during fasting, diminishes the action of hypoglycemic agents, and is necessary for the development of diabetes in all its intensity. An excess of anterior pituitary hormones increases diabetes or even brings about a diabetic state (even when the adrenals, thyroid, or pituitary are absent) which can be transitory (hypophyseal diabetes) or permanent (metahypophyseal diabetes). It greatly increases the resistance to insulin even when the pancreas is absent and causes a decrease in the endocrine secretion of the pancreas.

The lack of adrenal cortex brings about a gradual decrease of glycemia and glycogen in fasting. The intensity of pancreatic diabetes is markedly decreased. Cortical hormones correct all these disturbances and in high doses can produce a transitory diabetes. In dogs with reduced mass of pancreas, permanent diabetes (metacorticoid diabetes) can even be obtained. Adrenalectomy in man produces amelioration of glucose tolerance, or of diabetes and even cures some cases of diabetes in hypercorticalism.

The thyroid hormone increases intestinal absorption of glucose and rapidity of utilization of glycogen. Treatment with thyroid hormone does not produce diabetes in normal animals. In dogs with reduced mass of pancreas, thyroid treatment can produce transitory or even permanent diabetes. Thyroidectomy has a preventive and curative action on diabetes of subtotally pancreatectomized rats; it has no clear action on diabetes in dogs; it can diminish the intensity of diabetes in human beings.

Sex hormones have a definite action on the evolution of diabetes consecutive to subtotal pancreatectomy in the white rat. The ovary or estrogens diminish the frequency of this diabetes, the testicle or androgens increase its frequency and severity. A combined treatment with estrogens and insulin produces regression of a large proportion (65 per cent) of alloxan diabetes. Estrogens produce hyperplasia of islets and increase of insulin and also some action on pituitary and other glands.

In the endocrine equilibrium, any hormone can have synergic, antagonistic, conditioning, or regulating action with respect to other hormones.

In all forms of diabetes there is an insufficiency of insulin in relation to the needs of the organisms. The deficiency is absolute when there is lack of insulin secretion. The deficiency is relative (1) when the secretion is diminished; (2) when the secretion is normal, but the require-

ment of insulin is increased; and (3) when there is resistance to insulin.

In diabetes there is also an imperfect regulation of the endocrine secretion of the pancreas, since it cannot adjust itself to the needs of the organism in order to bring about a normal sugar level.

In all forms of diabetes, pituitary and adrenal secretions, whether normal or not, augment diabetes. All the endocrine glands play a part in all diabetic states, either directly because of their specific function or through their influence on other organs. The relative importance of these actions has not yet been established.

The various forms of experimental diabetes are due to the destruction of the normal equilibrium of all these factors. With time, it is hoped, it will be possible to establish what equilibrium exists in the various forms of diabetes met with in human beings. This knowledge should give us a firmer basis for the exact diagnosis and treatment of these cases.

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Chapter 19

INSULIN ANTAGONISM IN PLASMA

Philip J. Randle

A number of attempts have been made in recent years to detect insulin and insulin antagonists in the blood of diabetic patients or experimental animals. These investigations have been prompted partly by the need for a deeper understanding of the importance of insulin deficiency and insulin antagonism in the onset, persistence, and severity of diabetes mellitus in man; but more particularly because growth hormone (GH), which in vivo inhibits the uptake of glucose by diaphragm muscle and its response to insulin, does not have this effect on diaphragm in vitro. This has led to the idea that growth hormone may be transformed in vivo to an inhibitory substance and to attempts to identify such a substance in blood plasma. These experiments have involved comparing the effects of blood plasma from normal and diabetic animals on the uptake of glucose and response to insulin of isolated rat diaphragm or on the blood sugar response to insulin of suitable experimental animals. The present account will be largely restricted to a discussion of the interpretation and significance of results obtained with isolated diaphragm because it would seem that most future investigations will involve the use of this or similar in vitro systems.

EFFECT OF BLOOD PLASMA ON UPTAKE OF GLUCOSE BY ISOLATED RAT DIAPHRAGM

The uptake of glucose by isolated rat diaphragm is increased when insulin (at concentrations as low as 10^{-4} unit/ml.) or plasma from normal animals is added in vitro to the suspending medium. This response of isolated diaphragm to insulin forms the basis of an in vitro assay for insulin that has been used to estimate insulin in blood plasma. Its use in this connection depends upon the assumption that insulin is the only substance in plasma which affects glucose uptake. The effect of plasma on glucose uptake will depend upon the relative activities of substances such as insulin that enhance glucose uptake, and of substances that either inhibit glucose uptake (inhibitors), or diminish the effect of insulin, or other substances in plasma that increase glucose uptake (antagonists). The stimulating effect on glucose uptake of plasma from normal animals has been variously attributed to insulin in the plasma or to nonspecific effects of plasma protein. Attempts to distinguish between possible effects of insulin in plasma and of other plasma proteins on glucose uptake by investigating the effect on diaphragm of plasma from totally depancreatised animals have been largely frustrated because antagonists are present in the plasma of such animals. Furthermore, attempts to determine whether plasma proteins such as albumin can stimulate uptake of glucose have so far been unsuccessful because of the possibility that apparently pure plasma proteins such as albumin may be contaminated with significant amounts of insulin. More satisfactory evidence that appears to distinguish between these possibilities has been obtained by first identifying insulin in serum protein fractions by apparently reliable methods, followed by a study of the effect of these protein fractions on uptake of glucose by diaphragm. Since this problem is of the greatest importance to any interpretation of the significance of plasma inhibitors and antagonists these results are discussed below.

When I^{131}-labelled ox insulin of similar electrophoretic mobility to native ox insulin is added to normal human serum and the mixture subjected to electrophoresis on columns of treated cellulose, the labelled insulin migrates with a mobility slightly less than that of serum albumin (Fig. 19-1). If protein fractions (albumin; albumin + α-1-globulin; α-2-globulin; β-globulin; and γ-globulin; Fig. 19-2) prepared from normal human serum by zone electrophoresis on columns of treated cellulose are tested for ability to stimulate uptake of glucose by diaphragm, maximal biological activity is found in the slower-moving fraction of albumin (albumin + α-1-globulin). The faster-moving fraction

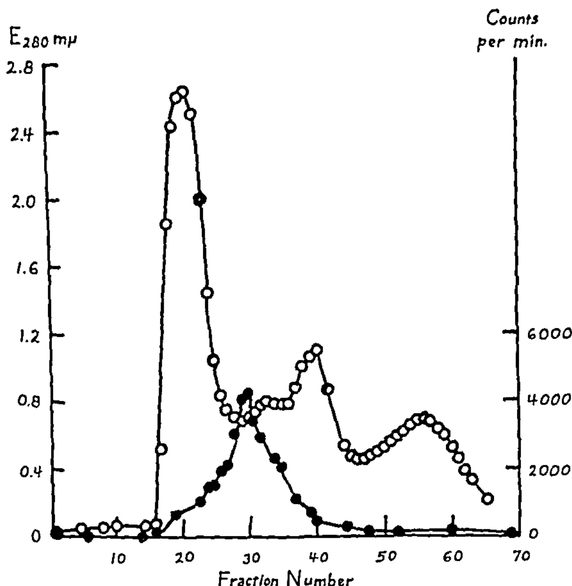


FIG. 19-1. Protein concentration and radioactivity of eluate from electrophoresis on columns of treated cellulose of a mixture of normal human serum and I^{131}-labeled insulin. o — o, protein concentration; • — •, radioactivity. (After Randle, P. J., and Taylor, K. W.: *J. Endocrinol. 17:*387, 1958.)

of albumin does not stimulate glucose uptake though some activity is found in β-globulin and γ-globulin fractions (Table 19-1). If unlabelled ox insulin is added to normal human serum before electrophoresis the activity of the albumin + α-1-globulin fraction is enhanced; that of other fractions is unaltered. These observations appear to show that serum albumin itself does not stimulate glucose uptake but that circulating insulin may contaminate serum albumin preparations and enable them to stimulate glucose uptake. The material present in β-

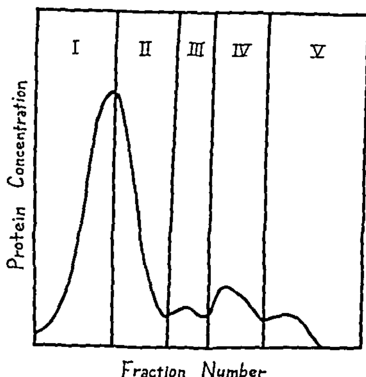


FIG. 19-2. Protein fractions obtained from normal human serum by electrophoresis on columns of treated cellulose. Fractions: I, albumin; II, albumin + α-1-globulin; III, α-2-globulin; IV, β-globulin; V, γ-globulin

globulin and γ-globulin fractions which enables them to stimulate glucose uptake may be insulin whose electrophoretic mobility has been altered by association with some other protein or it may be some sub-

TABLE 19-1. EFFECT OF PROTEIN FRACTIONS OF NORMAL HUMAN SERUM PREPARED BY ZONE ELECTROPHORESIS ON UPTAKE OF GLUCOSE BY ISOLATED RAT DIAPHRAGM

Added to incubation medium	*Glucose uptake. Mean ± S.E.M.* Mg glucose/Gm. of wet diaphragm/hr.*
No addition	2 3 ± 0 11
Fraction I (albumin)	2 3 ± 0 11
Fraction II (albumin + α-1-globulin)	3 4 ± 0 16
Fraction III (α-2-globulin)	2 3 ± 0 07
Fraction IV (β-globulin)	3 2 ± 0 16
Fraction V (γ-globulin)	3.5 ± 0 19

* Each mean is derived from 18 observations

stance other than insulin but with a similar effect on uptake of glucose by diaphragm. In what follows it will be assumed that most of if not all the stimulating effect of plasma on glucose uptake is due to circulating insulin.

INHIBITORS OF GLUCOSE UPTAKE AND INSULIN ANTAGONISTS IN PLASMA

EXPERIMENTAL DIABETES

Inhibitors and Antagonists of Pituitary-Adrenal Origin

Material capable of inhibiting the effect of insulin (or of serum albumin that is contaminated with circulating insulin) on uptake of glucose by diaphragm has been isolated in crude form from the plasma of alloxan-diabetic rats. The antagonist may be a β-lipoprotein since it is present in β-lipoprotein-rich fractions of serum prepared either by flotation on concentrated NaCl solution in the ultracentrifuge or by Cohn fractionation procedures and its activity is destroyed by repeated freezing and thawing. The antagonist appears to be formed from or under the influence of growth hormone and an adrenal steroid. Thus it could not be detected in plasma from alloxan-diabetic hypophysectomised rats (ADH rats) or alloxan-diabetic adrenalectomised rats or lipoprotein fractions therefrom, though it could be detected in plasma and lipoprotein fractions of plasma from ADH rats treated with GH and cortisone. Neither treatment with GH nor with cortisone alone restored the antagonist to the plasma of ADH rats nor was it restored when ADH rats were treated with one hormone and the other was added to the plasma in vitro. Furthermore, neither GH nor cortisone either alone or in combination affects either uptake of glucose or response to insulin of isolated diaphragm when added in vitro. Thus the antagonist is formed only in vivo and only when growth hormone and an adrenal steroid are available. Since lipoprotein-rich fractions of serum appear to be rich in steroids, it has been suggested that the antagonist may be a steroid-protein complex. There is at present no indication as to whether the antagonist is formed from growth hormone and adrenal steroid or is formed from other materials under their influence.

Material that antagonises the effect of insulin on diaphragm has been detected in the plasma of depancreatised cats. This antagonist does not appear to be a lipoprotein because it is not inactivated by repeated freezing and thawing. Preliminary fractionation studies suggest that it may be an α-2-globulin, a β-globulin, or γ-globulin. The antagonist ap-

pears to be formed from or under the influence of growth hormone and cortisol or cortisone. Thus it could not be detected in plasma from depancreatised-hypophysectomised or depancreatised-adrenalectomised cats and the effects of hormonal replacement, though incompletely investigated did appear to indicate that the antagonist was present only when growth hormone and an adrenal steroid were available.

The antagonists that appear to be present in plasma from alloxan-diabetic rats or depancreatised cats have yet to be isolated and characterised. They are significant insofar as they provide some explanation of the hormonal regulation of glucose uptake by muscle. It is generally conceded that uptake of glucose by isolated diaphragm is increased by the activity of endogenous insulin and diminished by the combined effects of endogenous GH and adrenocortical secretion. The effects of endogenous GH on the sensitivity to insulin of isolated diaphragm may be deduced from the results of experiments recorded in Table 19-2.

TABLE 19-2. HORMONAL REGULATION OF INSULIN SENSITIVITY OF ISOLATED RAT DIAPHRAGM

Source of diaphragm	*Concentration of insulin (Units/ml)*	*Effect of insulin on glucose uptake. Mean difference ± S E of difference, mg glucose/Gm of wet diaphragm*	
Normal rats	10^{-4}	2 1 ± 0 22 (23)	P* < 0 01
Hypox rats	10^{-4}	*2 9 ± 0 19 (24)*	
Normal rats	10^{-4}	1 4 ± 0 15 (58)	P < 0 05
Normal rats treated GH	10^{-4}	1 0 ± 0 13 (54)	
Hypox rats	10^{-3}	2 0 ± 0 06 (16)	*P < 0 05*
Hypox rats treated GH	10^{-3}	1 6 ± 0 14 (16)	

* *P, Probability of difference being due to chance. The number of observations is given in parenthesis* (unpublished observations of Manchester and Randle)

Diaphragms from hypophysectomised rats were found to be more sensitive to the effect of low concentrations of insulin (10^{-4} or 10^{-3} units/ml.) on glucose uptake than diaphragms from normal rats, diaphragms from normal or hypophysectomised rats treated with G[illegible] less sensitive to insulin than c[illegible]ing saline. It may [illegible]ed from these observations that [illegible] GH not only [illegible]ake of glucose by diaphragm m[illegible]t it also a[illegible]ct of insulin. Neither GH nor [illegible]ids, either [illegible]ir tion [illegible] affect the uptake of [illegible]onse to i[illegible]hragm

when added in vitro. Thus the antagonists which appear to be present in the plasma of alloxan-diabetic or depancreatised animals are important because they may represent the substance through which endogenous GH and adrenocortical secretion influence the uptake of glucose by muscle and its response to insulin.

The effects of plasma from rats and cats on the uptake of glucose by diaphragm and its response to insulin are summarised in Table 19-3.

TABLE 19-3. EFFECT OF PLASMA FROM RATS AND CATS ON UPTAKE OF GLUCOSE AND RESPONSE TO INSULIN OF ISOLATED RAT DIAPHRAGM

	Effect of plasma on isolated diaphragm		*Conclusions concerning presence in plasma of*	
Source of plasma	*Glucose uptake*	*Response to insulin*	*Insulin*	*Insulin antagonist*
Normal rat	Increased	Not affected	Present	Not detected
Alloxan-diabetic rat	Increased if antagonist removed or inactivated	Inhibited	Present	Present
ADH rat	Increased	Not affected	Present	Not detected
ADH rat treated GH + cortisone	Increased if antagonist removed or inactivated	Inhibited	Present	Present
Normal cat	Increased	Not affected	Present	Not detected
Depancreatised cat	Not affected	Inhibited	Not detected	Present
Depancreatised-hypox or depancreatised-adrex cat	Not affected	Not affected	Not detected	Not detected
Depancreatised-adrex treated cortisol or cortisone	Not affected	Inhibited	Not detected	Present

The results appear to show that plasma from normal rats and cats contains insulin and that the effect of insulin added to the plasma in vitro is not inhibited. After pancreatectomy in the cat no insulin could apparently be detected though the activity of an insulin antagonist was demonstrable. It is not clear from these results whether normal cat plasma is free of antagonist or whether the antagonist is rendered ineffective in the diaphragm test by insulin present in the plasma. Nor is it clear whether the antagonist can be detected in plasma from depancreatised cats because insulin is lacking or because the plasma level

of antagonist is increased. It would appear that insulin may be present in plasma from both normal and alloxan-diabetic rats, but that its effect on diaphragm is masked in the case of plasma from diabetic rats by the presence of antagonist. No antagonist could be detected in plasma from normal rats and it would appear that the plasma level of antagonist may be increased in the plasma of alloxan-diabetic rats, possibly because of hypersecretion of GH and an adrenal steroid.

Of Unknown Origin

Material capable of inhibiting the effect of insulin in the plasma on the uptake of glucose by diaphragm appears to be present in plasma from cats made permanently diabetic by treatment with GH. Since this antagonist is inactivated by freezing and thawing it may be a lipoprotein. Nothing is known of its relation to pituitary or adrenocortical secretions.

CLINICAL DIABETES

Diabetic Patients Without Insulin Resistance

The effects of plasma or protein fractions of plasma from normal people or diabetic patients on uptake of glucose by diaphragm and its response to insulin are summarised in Table 19-4. The results appear to indicate that insulin and an antagonist are present in plasma from normal people, that the activity of the antagonist cannot be detected in whole plasma; but that its activity can be demonstrated when it is separated from insulin by fractionation of plasma proteins with acid alcohol. Since the antagonist retained activity after the drastic fractionation procedure used, it is unlikely to be a lipoprotein. There is no indication as to the relation of this antagonist to pituitary or adrenocortical secretion, or to its nature or significance and it may merely represent a toxic substance formed as a result of the somewhat drastic chemical fractionation employed.

An insulin antagonist appears to be present in the plasma of insulin-requiring diabetics in whom the diabetes is poorly controlled. The antagonist but not insulin could be detected in whole plasma. When plasma from similar diabetic patients was fractionated with acid alcohol, insulin and an inhibitor of glucose uptake were detected in plasma protein fractions. Insulin was detected in whole plasma from insulin-requiring diabetics who were well controlled and an insulin antagonist could not be detected. Both insulin and an inhibitor of glucose uptake were demonstrable in protein fractions prepared with acid alcohol from the plasma of such patients. These results appear to show that the

TABLE 10-4. EFFECT OF PLASMA OR PLASMA FRACTIONS FROM DIABETIC PATIENTS ON UPTAKE OF GLUCOSE AND RESPONSE TO INSULIN OF ISOLATED RAT DIAPHRAGM

Source of plasma or plasma fraction	*Plasma or plasma fraction tested*	*Effect of plasma on isolated diaphragm*		*Conclusions*
		Glucose uptake	*Response to insulin*	
Normal people	Plasma	Increased	Not affected	Insulin present Antagonist not detected
Normal people	Plasma fractions 1. (Nature not known) 2.	Increased Depressed	Not tested	Insulin present Inhibitor present
Diabetic patients with ketoacidosis	Plasma	Depressed or not affected	Not tested	
Diabetic patients with ketoacidosis	Plasma fractions 1 (Nature not known) 2.	Increased or not affected Depressed or not affected	Not tested	Insulin and/or inhibitor present
Insulin-requiring diabetics well stabilised	Plasma	Increased	Not affected	Insulin present Antagonist not detected
Insulin-requiring diabetics well stabilised	Plasma fractions 1 (Nature not known) 2	Increased Depressed	Not tested	Insulin present Inhibitor present
Insulin-requiring diabetics, poorly controlled but not ketotic	Plasma	Not affected	Inhibited	Insulin not detected Antagonist present
Diabetics not requiring insulin, mainly obese	Plasma	Increased	Not affected	Insulin present Antagonist not detected

plasma of poorly controlled insulin-requiring diabetics contains both insulin and an antagonist. It would appear that the effect of insulin on isolated diaphragm is masked by the activity of the antagonist when whole plasma is tested. This may be because the plasma level of antagonist is increased or because the plasma level of insulin is reduced to a sufficiently low level for the activity of the antagonist to be detected. The plasma of insulin-requiring diabetics who are well controlled appears to contain insulin. It is not clear whether an insulin antagonist is present in the plasma of such patients, for the inhibitor that has been detected in protein fractions prepared from plasma with acid alcohol may be an artefact induced by the fractionation procedure employed. The plasma of those diabetics, mainly obese, who do not require insulin appears to contain insulin. No antagonist could be detected in the plasma of such patients. This does not necessarily mean that an antagonist was not present for the plasma may have contained an amount of insulin sufficient to mask the activity of the antagonist. The antagonist that has been detected in the plasma of poorly controlled insulin-requiring diabetics does not appear to be a lipoprotein, for it is not inactivated by repeated freezing and thawing. There is no indica-

tion as to whether the antagonist is formed from or under the influence of GH and cortisol, though it is interesting to note that it could be detected in samples of diabetic plasma which contained normal amounts of 17-hydroxycorticosteroids. This antagonist does not appear to be an antibody to injected heterologous insulin, for it was detected in the plasma of diabetic patients who had never received insulin and it does not appear to be a γ-globulin. This antagonist may well be a significant factor in the diminished sensitivity to insulin which poorly controlled diabetics are known to show. Failure to demonstrate an insulin antagonist in the plasma of obese diabetics who do not require insulin may mean either that the diminished sensitivity to insulin which is a feature of such cases does not result from the activity of a humoral antagonist to insulin or alternatively that the experimental conditions necessary for the detection of an antagonist were not achieved.

Diabetic Patients with Insulin Resistance

The occasional occurrence of insulin resistance in diabetics necessitating the daily administration of several hundred or thousand units of insulin is well known. Insulin has been shown to be weakly antigenic and in many cases a circulating antibody may be responsible for the insulin resistance. In such instances manifestations of allergy may be present and the serum or γ-globulin fractions prepared therefrom may be capable of neutralising biologic effects of insulin. This aspect of serum factors that modify insulin action will have been considered more fully elsewhere and need not be mentioned further here. However, in some cases of insulin resistance an antagonist to insulin has been detected in serum that does not appear to be an antibody. The patients studied were ketotic diabetics with insulin resistance, i.e., requiring several hundred or thousand units of insulin daily. The antagonist was detected in plasma by its ability to prevent the stimulating effect of insulin on glycogen synthesis by isolated rat diaphragm in vitro. The antagonist, which could only be detected in the plasma of those ketotic diabetics exhibiting insulin resistance, disappeared from the plasma when the patient was adequately treated with insulin and the resistance to insulin overcome. The origin of this antagonist is at present obscure. It does not appear to be an antibody because it was detected in the α-globulin fraction of serum. Insofar as it could not be detected in the plasma of diabetic patients, some with ketosis, with an abnormally high plasma level of cortisol, the antagonist may not be formed from or under the influence of cortisol. The relationship between this antagonist and the activity of growth hormone is not yet clear. The antagonist could not be detected in the plasma of an acromegalic with

insulin-resistant diabetes but this does not mean that the antagonist was not present. There is evidence that the plasma level of insulin may be increased in acromegaly even when diabetes coexists. The relative levels of insulin and antagonist in plasma may be of the greatest importance to the detection of an antagonist in the test employed and the presence of additional insulin in acromegalic plasma might well prevent the detection of an insulin antagonist.

From the evidence presented and particularly because of the apparent correlation between the presence and absence of insulin resistance and of the antagonist in the plasma, it would appear that this antagonist may be a significant factor in the insulin resistance encountered in some cases of diabetes with ketoacidosis.

Inhibitors and Antagonists in Plasma from Nondiabetic People

It is generally conceded that endogenous growth hormone and adrenal steroids influence sensitivity to insulin in normal animals, including man. Furthermore, the results recorded in Table 19-2 appear to show that endogenous GH affects the sensitivity to insulin of rat diaphragm muscle. Thus it would seem that an insulin antagonist must be present in plasma from normal animals. There is no convincing direct evidence on this point at the present time, principally, one suspects, because the right experimental conditions have not been achieved. Plasma from normal people does not inhibit the effect of insulin on isolated diaphragm when both are present in vitro but plasma from normal people probably contains a quantity of insulin sufficient to mask the effects of an antagonist. There is some evidence from studying the effect of different dilutions of plasma on uptake of glucose by diaphragm that a substance is present which inhibits the effect of circulating insulin on diaphragm and loses its effect when plasma is diluted. Furthermore, in some instances it has been possible to obtain an α-2-globulin fraction from normal human serum that inhibits glucose uptake. However, this is not a consistent finding and in other instances α-2-globulin fractions have been obtained that either stimulate or do not affect glucose uptake. Nevertheless, the author is convinced that more refined methods of plasma protein fractionation will reveal an insulin antagonist in plasma from normal animals.

Plasma from Psychotic Patients with Insulin Hypoglycaemia

β-Lipoprotein-rich fractions of serum from psychotic patients during insulin hypoglycaemia have been shown to reduce the stimulating effect of serum albumin on uptake of glucose by diaphragm. Since the stimulating effect of serum albumin appears to be due to insulin present in

preparations of this protein, it may be inferred that an insulin antagonist was present. It is known that sensitivity to insulin may be diminished following insulin hypoglycaemia and the antagonist which appears to be present in serum during insulin hypoglycaemia may be of significance in this connection.

SUMMARY AND CONCLUSIONS

It would appear that insulin antagonists of significance in diabetes have been demonstrated in blood plasma. Judged from the purely experimental standpoint, the attack on this problem appears to have been piecemeal, principally, one suspects, because the nature of the problem and the limitations of existing techniques are only now being defined. The detection either of insulin or of an insulin antagonist in plasma by purely biological methods is clearly dependent upon the relative levels of each factor. Thus it would appear that excess of insulin may prevent the detection of an antagonist in plasma and vice versa. In future work fractionation procedures will have to be devised that can separate plasma insulin from insulin antagonists. When such procedures are available, it may then be possible to detect insulin antagonists in blood from normal animals or in blood from animals with an intact pancreas and treated, for example, with growth hormone or adrenal steroids. It would seem that the detection of insulin and insulin antagonists in protein fractions of plasma will depend upon biologic methods for many years. Such methods will almost certainly involve the use of isolated tissues such as diaphragm, for in vivo methods are too laborious and too difficult to standardise on the scale that will be necessary when attempts are made to isolate antagonists from plasma in pure form.

So far no mention has been made of possible mechanisms of action of insulin antagonists. The antagonist that has been identified in the serum or γ-globulin fraction of serum from insulin-treated or insulin-resistant diabetics and which appears to be an antibody may act by combining with insulin in such a way as to render it biologically inactive. We do not know whether other insulin antagonists have a similar effect, but if they do, then ordinary methods of plasma protein fractionation such as electrophoresis may not be capable of separating insulin from insulin antagonists. Special methods of dislocating possible complexes of insulin and antagonist may have to be devised before both can be detected in serum from normal animals.

In considering all the insulin antagonists detected in plasma it would appear that three types have been identified. One appears to be an antibody which combines with heterologous insulin and which is located

in the γ-globulin fraction of serum from diabetic patients, with or without insulin resistance, who have been treated with heterologous insulin for several months. The possibility also exists, though satisfactory evidence is at present lacking, that there may be a similar factor in the γ-globulin fraction of serum from normal animals and which combines with homologous insulin. Another antagonist, which appears to be a lipoprotein, has been detected in the serum of alloxan-diabetic rats, metahypophyseal diabetic cats, and psychotic patients with insulin hypoglycaemia. There is some evidence that this inhibitor is only to be found in plasma when both growth hormone and an adrenal steroid are present. The interesting possibility exists that this antagonist is a complex formed between growth hormone and an adrenal steroid. Since it has only been detected in the serum of animals in which much of the pancreas is present, it might be formed in that organ. The third type of antagonist, possibly an α-globulin, has been detected in plasma from depancreatised cats, poorly controlled insulin-requiring diabetic patients, and insulin-resistant diabetic patients with severe ketoacidosis. There is evidence that this inhibitor is also formed from or under the influence of growth hormone and an adrenal steroid. The real significance of the putative lipoprotein and α-globulin type of inhibitors cannot be assessed until they have been isolated and their exact relationship to growth hormone and adrenal steroids defined. Nevertheless, it would appear that both poor control in diabetes and insulin hypoglycaemia are associated with a rise in the blood level of an insulin antagonist and possibly therefore with increased secretion of pituitary growth hormone and adrenal steroid. This may contribute significantly to the instability of some diabetics (brittle diabetics) and possibly to the development of retinal, renal, and cardiovascular complications in poorly controlled diabetics.

The failure to identify an insulin antagonist in plasma from diabetics, mainly obese, who do not require insulin but nevertheless show insensitivity to the hypoglycaemic action of insulin emphasises that insulin antagonism may not always be humoral in origin. Presland and Todd have recently reported the results of their investigation of an insulin-resistant diabetic requiring several thousand units of insulin daily. The serum and protein fractions of serum contained sufficient insulin to cause hypoglycaemic convulsions on injection into experimental animals and no antagonist could be detected in the serum or protein fractions therefrom. Their elegant experiments appear to show conclusively that the cause of insulin resistance is not always to be found in the plasma of diabetic patients and that insulin resistance may sometimes originate in a change in the cells themselves.

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Chapter 20

IMMUNOLOGIC REACTIONS TO INSULIN

Solomon A. Berson and Rosalyn S. Yalow

Immune responses to insulin in man may be manifested clinically in two forms, allergic reactions and relative resistance to the hypoglycemic effects of insulin. Occasionally insulin allergy and insulin resistance coexist in a single subject. However, most patients with insulin allergy do not exhibit obvious resistance to insulin and the disappearance or lessening of skin sensitivity subsequent to the development of insulin resistance has been reported. Whereas sensitivity to insulin preparations is recognized readily, the demonstration that insulin resistance is due to immune mechanisms may be a difficult task since any of a number of other factors, the presence of which cannot always be excluded, may modify the hormonal effects of insulin.

INSULIN ALLERGY

Untoward responses to insulin may be local or general. Dermal reactions at the site of injection, characterized by burning or itching followed within a few hours by erythema and induration, occur frequently, with an incidence reported as high as 55.8 per cent in a carefully

studied group (14). Sensitivity of this type is exhibited during the first or second week following institution of insulin therapy and generally disappears after a few weeks or months of continuous treatment but occasionally persists for as long as a year or more. A question frequently raised is whether such reactions are due to insulin itself or to contaminating proteins or other substances. In the first few years following the introduction of insulin therapy, the relatively crude preparations employed not infrequently produced systemic reactions that generally took the form of a mild serum sickness (17). Furthermore, it is common experience that modified insulins such as protamine zinc insulin and globin insulin are more frequent offenders than regular insulin. In some cases sensitivity to the preservatives or acid buffers employed in insulin solutions was thought to be responsible for reactions but this has been denied (13). A decrease in the severity of reactions in sensitive persons has been observed frequently on changing to recrystallized insulin and Jorpes (8) reports that diabetic patients sensitive to commercial insulin preparations are entirely free of reactions when taking mixtures of beef or pork insulin that have undergone repeated recrystallization. Dolger has found that merely boiling insulin, which does not abolish its hormonal activity, destroys the dermal sensitizing factor (5). It seems probable, therefore, that most local manifestations of so-called insulin sensitivity are due to the presence of other materials.

Since local sensitivity usually disappears spontaneously within a few weeks or months, special therapeutic measures are not indicated in mild cases although antihistamine agents may be beneficial in the control of symptoms. In more severely reacting subjects, recrystallized or boiled insulin preparations should be employed.

Much more rarely, generalized reactions occur in the form of urticaria, angioneurotic edema, or even anaphylactic shock. Such reactions most commonly follow shortly the resumption of insulin therapy after a period of omission and, in many cases at least, appear to be instances of true insulin allergy. Highly sensitive subjects frequently show cutaneous and ophthalmic reactions to minute amounts of insulin and their sera may contain passive transfer antibodies capable of sensitizing normal skin to insulin. Skin-sensitizing antibody is destroyed by heating (12).

Successful treatment of persistent insulin allergy involves cautious desensitization. If severe diabetic acidosis is present, a rapid desensitization regime may be indicated as an emergency procedure. Repeated injections beginning with one five-hundredth unit to one one-thousandth unit and doubling the dose every 15 to 30 minutes may effectively desensitize within a matter of hours. Adrenocortical steroids may be employed temporarily to protect against severe reactions. During the

course of desensitization, the development of thermostable antibodies, which are capable of "blocking" the wheal effects of insulin on sensitized skin of normal subjects, has been reported (10). Blocking antibody has also been found in the sera of nonsensitive, insulin-resistant subjects and appears to be distributed between both fast and slow γ-globulins separated by electrophoresis convection (11).

INSULIN RESISTANCE

The criterion for insulin resistance generally accepted is the persistent requirement of more than 200 units of insulin per day for satisfactory control of blood sugar. However, in view of the much lower needs of completely depancreatized subjects, it would seem reasonable to regard a requirement of anything more than about 100 units per day as evidence for some degree of insulin "hyporesponsiveness."

In view of the large number of diabetic patients who depend on insulin, *the proportion maintaining a requirement in excess of 200 units* daily is small indeed. Smelo (16) was able to collect only 54 such cases, due to all causes, from the English literature to 1948 and in only 9 of these did accompanying local or general allergy permit their classification under the heading of "immunologic inactivation." Yet the condition is surely much more common than these figures would indicate. In the author's laboratory alone the sera of 9 patients with insulin resistance of immune origin have been studied over a two-year period. Recent evidence indicates that virtually all insulin-treated subjects develop circulating antibodies against insulin (1) but the plasma concentration of such antibodies ordinarily is so low that detection is possible only with very sensitive techniques. Insulin resistance in a clinically recognizable form develops only in the rare patient who produces extraordinarily high concentrations of antibody. Nevertheless, because of the almost ubiquitous antibody response to insulin therapy, the concept that some degree of subclinical resistance exists in most insulin-treated patients not only is tenable from a strictly academic point of view, but also serves to explain the oft-observed hyporesponsiveness of many such patients to small test doses of insulin.

In early studies the development of resistance was shown to be associated occasionally with the presence of a serum factor capable of antagonizing the hypoglycemic effects of insulin in animals. In some cases the coexistence or past history of insulin allergy suggested that the insulin antagonist was a neutralizing antibody. Subsequently, insulin-neutralizing antibodies were reported to accompany the γ-globulins on salt fractionation (3, 4) or starch electrophoresis separation (15) of the

serum proteins. In earlier studies, positive precipitin reactions were reported in some cases of insulin resistance, but more recent investigations have been unsuccessful in demonstrating precipitability of insulin-antibody complexes. From recent evidence concerning the nature of insulin antigenicity it appears possible that insulin itself forms only soluble complexes with antibody and that the precipitin reactions observed were due to contaminating proteins or resulted from alterations of insulin during extraction.

Because of the paucity of cases in which insulin antibodies had been demonstrated and the difficulty experienced in immunizing animals to insulin, the hormone has generally been regarded as being poorly antigenic. Successful immunization of animals with insulin has been reported by several groups of workers but, in general, the use of adjuvants or alum precipitation has been required to render insulin antigenic. In man globulins capable of binding insulin can almost always be demonstrated after 3 to 4 months of continuous insulin therapy and frequently as early as 4 to 5 weeks following the first insulin injection. The uniform absence of insulin-binding globulin in the circulation of nontreated subjects and its appearance during the course of insulin therapy in diabetic subjects or nondiabetic schizophrenic patients attest to its antibody character.

Since the insulin-binding capacity of such serums is generally limited to only small quantities of insulin, it is necessary to employ tracer amounts of insulin labeled with I^{131} for detection of the antibody. The phenomenon of insulin binding is most readily demonstrated by means of paper electrophoresis or chromatography. Advantage is taken of the fact that insulin adsorbs rather firmly to certain inert substances like paper and glass. Adsorption to paper is not inhibited by the presence of small amounts of plasma so that if normal serum or plasma to which insulin-I^{131} has been added is applied to paper and the serum proteins are caused to migrate by electrophoresis or chromatography, the radioactive insulin remains at the site of application ("origin") (Fig. 20-1). In contrast, when serum from insulin-treated subjects is employed, some or all of the insulin-I^{131} is found to migrate just in advance of the γ-globulins, suggesting that insulin is bound to proteins in that region (Fig. 20-1). Insulin binding to serum globulins in these cases is demonstrated also by means of starch block electrophoresis and ultracentrifugation.

Whereas insulin-I^{131} escapes rapidly from the circulation of control subjects and is metabolically degraded at a rate of about 2 per cent per minute (1), there is prolonged retention of insulin-I^{131} in the circulation of insulin-treated subjects as a direct result of its binding by serum

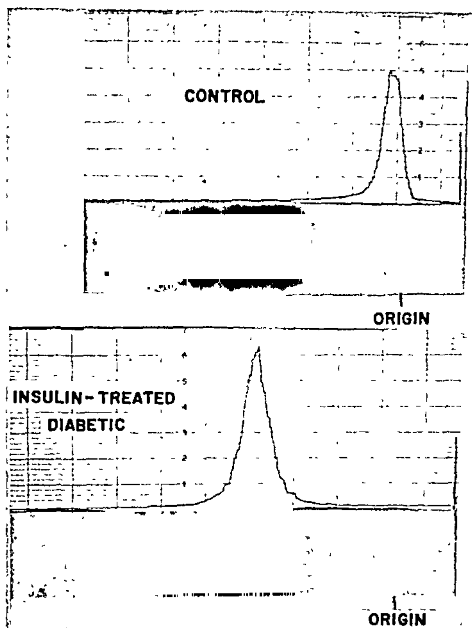


FIG. 20-1. Paper radioelectrophoretograms of insulin-I^{131} in plasma of a control subject (*upper*) and serum of an insulin-treated subject (*lower*).

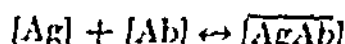
globulins. The insulin-globulin complex cannot, because of its relatively large size, readily penetrate the capillary wall and escape from the blood stream is much delayed. Furthermore, even after passage into extravascular spaces, the binding to globulin must restrict the access of insulin to cells and result in a marked retardation of the rate of insulin degradation. In vitro studies have demonstrated that insulin bound to

antibody is protected against proteolytic destruction by insulinase derived from liver homogenates (19). The protection of mice against the hypoglycemic effects of insulin administered together with plasma from insulin-treated subjects was correlated with marked retention of insulin-I^{131} in the circulation of the donors (18) and high concentrations of insulin-binding antibodies were found in the sera of insulin-resistant patients previously observed to possess insulin-neutralizing antibodies by the mouse convulsion test (2). These observations offer support for the identity of insulin-binding and insulin-neutralizing antibodies.

Inasmuch as the development of insulin-binding antibodies is a characteristic response to insulin therapy, it might be questioned why insulin resistance is not more commonly encountered. The explanation appears to lie in the type of antigenicity exhibited by insulin. Antibody formation in man is almost always stimulated by insulin but generally only to a slight extent. Although binding capacities of 20 to 25 units per liter are occasionally observed in the absence of definite clinical evidence of insulin resistance, the majority of insulin-treated diabetic subjects have plasma concentrations of antibody sufficient to bind at most some 10 units of insulin per liter plasma. In these patients, therefore, a relatively small amount of insulin is sufficient to saturate completely the antibody-combining sites; insulin in excess is free to act in the same manner as in nonimmune subjects. In insulin-resistant patients, however, the insulin-binding capacities encountered are considerably greater, ranging from 60 units per liter to over 1,000 units per liter plasma. Not only are relatively enormous doses of insulin necessary to satisfy all the available antibody-combining sites in circulation but it must be supposed also that continued antibody production creates constant demands for large amounts of insulin. Although insulin that becomes complexed to antibody can dissociate therefrom and gain access to tissue cells, some of the insulin within the complexes may be metabolically degraded with the antibody molecules without ever having been able to exert a hormonal effect. Since antibody globulin has an apparent space of distribution of about 7 liters and is turned over at a rate of about 4 per cent per day in the average man, several hundred units of insulin may be wasted daily simply in keeping up with antibody turnover if insulin-antibody complexes are metabolized at the same rate. However, it is conceivable that the soluble insulin-antibody complexes are subject to more rapid disposal and degradation than is the uncomplexed antibody.

Kinetic studies of the insulin-antibody reaction have shown that the formed complex is in reversible equilibrium with free insulin and free

antibody. The reaction may be pictured as obeying the law of mass action according to the following,



$$[Ag] + [Ab] \leftrightarrow [\overline{AgAb}]$$

where [Ag], [Ab], and $[\overline{AgAb}]$ represent the molar concentrations of insulin, antibody, and antigen-antibody complex respectively. These studies have shown that the ratio of bound to free insulin in any particular antiserum is inversely related to the concentration of insulin (Fig. 20-2). Thus, with increase in insulin concentration the ratio de-

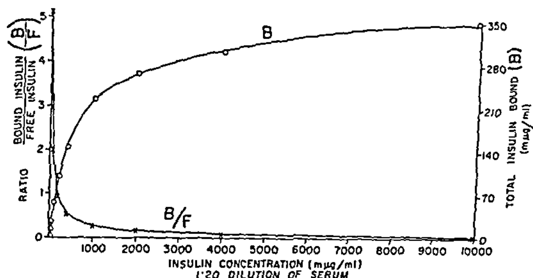


Fig. 20-2. Ratio $\frac{\text{bound insulin}}{\text{free insulin}}$ *and concentration of bound insulin as a function of total insulin concentration in serum of insulin-resistant subject*

creases although the absolute amount of bound insulin increases until the antibody-combining sites are completely saturated (Fig. 20-2). Insulin appears to be univalent in these reactions, combining with but a single molecule of antibody, which explains the lack of precipitation of the insulin-antibody complexes. At least two types of complexes exist, one of which dissociates rapidly with a half time of 3 to 15 minutes, the other much more slowly with a half time of 2 to 24 hours.

The in vitro studies help to picture the behavior of these soluble complexes in vivo. At low concentrations of insulin relative to the combining capacity of the antiserum, the insulin that dissociates from the antigen-antibody complex finds many vacant antibody sites with which it can again combine rapidly so that the ratio of bound to free insulin is maintained at a high level. In the same serum at much higher insulin

concentrations a large fraction of the antibody-combining sites are saturated so that insulin molecules which dissociate are more likely than not to escape into tissue sites before recombining with antibody.

Subjects in whom the antibody concentration has increased rapidly for one reason or another may develop, within a relatively short period of time, obvious loss of blood sugar control and require hospitalization for diabetic ketosis. It is soon recognized that the patient is insulin-resistant and several thousand units may be administered during the first day or two before acidosis and glycosuria are brought under control. Frequently, however, insulin requirements fall to zero by the next day and recurrent episodes of hypoglycemia may be experienced for several days thereafter. It must be supposed that the high initial insulin dosage had resulted in the trapping of a large amount of insulin in insulin-antibody complexes. Subsequent to the restoration of normal blood sugar levels, the slow release of insulin from the dissociating complexes can provide the tissues with a continuing supply of insulin sufficient to induce reactions.

It is no more known why some patients produce relatively large amounts of insulin-binding antibody than why some individuals are particularly good manufacturers of antibodies to other antigens. The possibility that infections in diabetic subjects might enhance, non-specifically, the synthesis and release of insulin antibodies was suggested earlier by Lerman (9) but supporting quantitative evidence on this point is lacking.

The specificity of insulin-binding antibody is of interest in relation to the structure of insulin. From Sanger's studies of beef, pork, sheep, whale, and horse insulins, it has been determined that in all species the amino acid sequences are identical except for positions 8, 9, and 10 in the glycyl (A) chain (7). If it can be supposed that human insulin is likewise unique only in one or more of these positions, then the antigenic site must be considered most likely to reside in this region. In support of the concept that positions 8 to 10 of the A chain are important in the antigenicity of insulin are observations from the authors' laboratory that indicate marked differences in the degree to which crystalline beef, pork, and horse insulins labeled with I^{131} react with the antiserum from human subjects treated with beef, pork insulin mixtures. At the same insulin concentrations, beef insulin is bound to a much greater extent than is pork or horse insulin. These differences in reaction can be attributed only to the known differences in the insulin molecules in the 8, 9, and 10 positions. These observations are also consistent with the univalency of insulin in these reactions, since even if antibodies of different specificity were directed against the individual amino acids

in the 8, 9, 10 positions, it would not be sterically possible for more than a single antibody molecule to react at this small site.

Human insulin cross-reacts with human antibodies to beef, pork insulin but to a much less extent than does any of the three animal insulins mentioned (Fig. 20-3). The cross reactions observed with these recrystallized insulin preparations from different species and especially

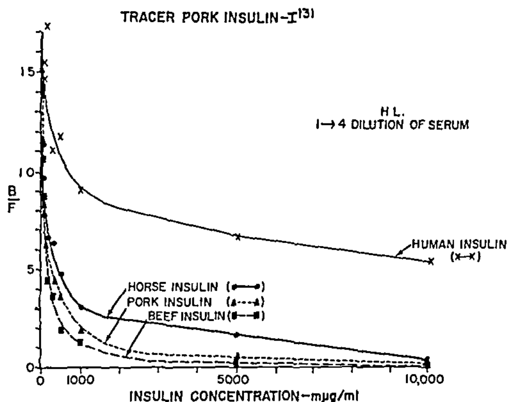


FIG. 20-3. Ratio $\frac{\text{bound pork insulin-I}^{131}}{\text{free pork insulin-I}^{131}}$ as a function of concentration of added beef, pork, horse, and human insulins.

the ability of human insulin to react are positive evidence that the antibody is directed against insulin rather than against contaminating animal tissue proteins. *Fortunately, the binding of human insulin is* so much weaker than the binding of animal insulins that significant resistance toward endogenous human insulin in vivo would not generally be anticipated. Lowell (12) had earlier shown that a subject resistant to the hormonal effects of beef, pork insulin still responded to the administration of human insulin. As emphasized by Lerman (9), this did not exclude completely the cross reactivity of human

insulin but indicated only a lesser degree of reaction. Human insulin is capable of eliciting positive dermal reactions (6) and even severe generalized urticaria (12) in sensitive subjects, indicating its ability to react with sensitizing antibodies produced in response to commercial insulin preparations.

The treatment of insulin resistance is a knotty problem. The patient who requires 500 units of insulin per day is deriving the hormonal benefits of only some 10 per cent of this amount, the remainder being disposed of through abnormal mechanisms. Since a relatively small change in the latter may result in a halving or doubling of the amount of insulin available for hormonal action, the difficulty in steering accurately between the Scylla of diabetic coma and the Charybdis of hypoglycemic shock is not surprising. The resistant state may persist for some years but in the absence of a suitable substitute for insulin, insulin therapy must be continued. Patients who have sufficient endogenous insulin to resist acidosis, if not severe hyperglycemia and glycosuria, may benefit from the use of the hypoglycemic sulfonylurea agents but in other cases these drugs are unlikely to achieve any success in the treatment of insulin resistance. Adrenocorticotropic hormone may produce a significant reduction in insulin requirements but is frequently without effect.

It must be hoped that an altered insulin can be prepared in which the hormonal effects are retained while the capacity for reacting with antibody is abolished. Even though the alteration may in itself result in the appearance of a new antigenicity, such a preparation would, in any event, find usefulness as a temporary expedient in difficult cases.

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Chapter 21

DEGRADATION OF INSULIN

Henry H. Tomizawa

Parenteral inactivation of insulin was demonstrated shortly after the discovery of this hormone in 1922. However, until recently this process of insulin inactivation was sporadically investigated and consequently was poorly understood. In 1947, Mirsky and co-workers began an extensive investigation of this process. In these studies, the degree of inactivation of insulin upon its incubation with various tissues was measured by the resultant decrease in hypoglycemic effect of a given quantity of insulin. Based on the results of these and subsequent experiments, Mirsky proposed that diabetes mellitus may be caused by an excessive rate of destruction of insulin by an enzyme, insulinase.

The report by Ferrebee and co-workers of the preparation of I^{131}-labeled insulin with no detectable loss of biological activity encouraged several groups to investigate the feasibility of using lightly iodinated insulin for studying the metabolism of this hormone. On the basis of studies with I^{127} (stable iodine), it is presumed that I^{131} is substituted for hydrogen at one or more of the 3 and 5 positions of the four tyrosine residues in insulin. It thus becomes an externally labeled compound, one in which a foreign atom is chemically attached. This differs from an internally labeled compound in which a radioactive atom has replaced a stable atom of the same element, i.e., glucose-C^{14}.

Although, with insulin-I^{131}, direct removal of I^{131} is only one of several possibilities that might lessen the suitability of insulin-I^{131} for tracer studies, use of this compound has certain advantages. Radioiodination of insulin is accomplished rather simply. This is in marked contrast to the preparation of insulin-C^{14}, which has not been made with sufficiently high specific activity. In addition, since I^{131} is a relatively strong gamma-ray emitter, a minimum of sample preparation is needed before counting. This had not been the case with C^{14}-labeled compounds until the recent introduction of liquid scintillation counting.

DEGRADATION OF INSULIN IN VIVO

The main difficulty in studying the inactivation of insulin in intact animals after administering native (unlabeled) insulin is that of detection. The activity of exogenous insulin has been demonstrated only in blood. For this purpose, biologic assays are used. The usefulness of these assays is limited, since processes other than degradation also decrease insulin activity. In addition, there is very little likelihood that in vivo degradation products of even large quantities of native insulin can be detected by the techniques now available.

In efforts to demonstrate participation of the liver in inactivating insulin, comparisons have been made between the hypoglycemic effect after insulin injection into the portal vein and the effect after insulin administration into a peripheral vessel. The measure of biologic activity has been either by the hypoglycemic response of the experimental animal itself or of a test animal injected with blood from the experimental animal. The majority of results of such experiments support the concept that the liver inactivates insulin in vivo.

Inactivation of insulin by kidney in intact animals has been reported by Gambassi and Giorgio, who found that the biologic activity of insulin introduced through a cannulated renal artery was not detected in blood collected through a cannula in the corresponding renal vein.

Most of these studies with native insulin have therefore suggested participation of certain organs in the inactivation of insulin. However, such studies do not reveal the mode of this inactivation. Included among the possibilities are simple removal of insulin by an organ, neutralization of insulin through binding by some factor produced in such an organ, and degradation of insulin

Most of the recent information concerning the inactivation of insulin has been obtained using insulin-I^{131}. Results from early experiments on the distribution of insulin-I^{131} in humans, as well as in intact animals, suggest that insulin-I^{131} is rapidly degraded by processes involving many

tissues. Insulin-I^{131} is almost completely precipitable with trichloroacetic acid (TCA) and is nondialyzable under suitable conditions. Yet, after injection of this preparation, there is a rapid rise in TCA—nonprecipitable radioactivity within many organs and an early urinary excretion of dialyzable radioactivity. Also, iodinated fragments with mobilities different from insulin-I^{131} have been demonstrated by means of paper electrophoresis. The observation that insulin and insulin-I^{131} compete equally in vivo in saturating the degradative systems indicates that the breakdown of insulin-I^{131} is a reflection of the degradation of native insulin. The question as to whether or not the degradation of insulin-I^{131} results in a concomitant loss of its biologic activity was recently investigated in eviscerated-nephrectomized rabbits by Drury and his co-workers. With animals so prepared in which insulin-I^{131} degradation is sufficiently slowed, there was a good correlation between the decrease in biologic activity of the injected insulin-I^{131} with the decrease in TCA-precipitability of radioactivity. Also using eviscerated-nephrectomized rabbits, Karasek and co-workers have suggested that the degradation of insulin-I^{131} is not necessarily a function of the action of insulin on target organs.

Several factors influence the extent of degradation of insulin-I^{131}. Nephrectomy and hepatectomy decrease its degradation: the catabolic role of the kidney and liver is again implicated. Hypophysectomy also depresses insulin-I^{131} degradation and it is thought that this may contribute to the insulin hypersensitivity of hypophysectomized animals. Insulin-I^{131} degradation is increased by thyroxin and decreased by thyroidectomy; these observations are consistent with findings concerning the effect of thyroid function on insulin requirements in diabetes mellitus. Another factor that decreases the degradation of insulin-I^{131} is insulinase-inhibitor, a substance partially purified from liver. It has been proposed that this material, probably a peptide, may regulate the amount of "insulinase" available for the destruction of insulin.

An interesting finding is that the rate of insulin-I^{131} degradation while normal in untreated diabetics, is slowed in persons who have received insulin injections for several weeks or more. This was found to be the case with schizophrenics treated by insulin shock, as well as with treated diabetics. The decreased degradation has been attributed by Berson and Yalow to the binding of insulin-I^{131} by an acquired antibody which, complexed to insulin, migrates to the inter-γ-β region upon electrophoresis.

It is likely that some of the information obtained concerning the metabolism of insulin-I^{131} will subsequently prove to be inapplicable to the metabolism of native insulin. The possibility of deiodination of insulin-I^{131} has been mentioned; there also may be deiodination of its

partial degradation products. Introduction of the large I^{131} atom may alter the structure of insulin because of steric and charge effects. On the basis of work by Yalow and Berson, radiation damage must be considered. Finally, there may be some loss of biologic activity that cannot be detected by bio-assay. The above possibilities notwithstanding, results already obtained have demonstrated that tracer studies with insulin-I^{131} can be of value. Equally important, these studies have created considerable interest in insulin distribution and degradation, areas formerly neglected for lack of suitable methods.

The relatively good success with insulin-I^{131} should give impetus to extensive experimentation with other modified insulins.

One such compound is fully biologically active guanidinated insulin, which has recently been prepared by Evans and Saroff. This is insulin in which the *Σ-amino group of lysine is completely converted to a Σ-guanidino group* (1 mole Σ-guanidino/1 mole insulin), and one-half of the α-amino group of the N-terminal glycine is guanidinated (0.5 mole α-guanidino/1 mole insulin). As Geschwind and Li suggest, introduction of C^{14} when preparing the guanidinated derivative should yield a modified insulin that would be useful for tracer studies. It would be interesting to learn to what extent studies with guanidino-C^{14} insulin correlate to those using insulin-I^{131}. In addition, long-term experiments might be performed which were not feasible with insulin-I^{131} because of the 8-day half-life of I^{131}.

DEGRADATION OF INSULIN IN VITRO

The ability of preparations of many tissues to inactivate insulin is well established. Much of the work concerning characterization of the system for insulin inactivation has been performed with liver preparations. Liver is very active in this respect in vitro, and as mentioned earlier, may play a similar role in vivo. In earlier investigations, the bio-assay was the sole method available for determining insulin inactivation. From studies using only bio-assays for detection, it was learned that insulin inactivation by liver involves participation of a heat-labile system that has optimal activity in the neutral pH range. Since this system is inhibited by reagents which react with sulfhydryl groups, it was postulated that this is an enzymatic system in which sulfhydryl groups are essential. Although these studies did not yield direct evidence as to the mode of the inactivation process, it was gen[illegible] that one or several of the proteolytic [illegible] prevalent [illegible] well as in many other tissues might be [illegible]

Since results of stu[illegible] I^{131} suggests

that insulin is degraded in its inactivation, the suitability of insulin-I^{131} for studying this process with liver preparations was determined. Upon 37° C. incubation of insulin-I^{131} with liver extract at pH 7.5, there is a rapid rise in TCA-soluble radioactivity. Here, as in the case of in vivo studies, the possibility of direct deiodination of insulin-I^{131} was of primary concern. Several observations indicate that liver preparations do not cause direct removal of I^{131} from iodinated insulin. Incubation of certain other I^{131}-labeled proteins under similar conditions does not give rise to TCA-soluble radioactivity. This suggests that a deiodinase for iodoproteins does not exist in the liver. Also, native insulin can be added in excess of the amount necessary to saturate the enzyme system, and thereby depress the rate of degradation of insulin-I^{131}. This suggests that insulin and insulin-I^{131} are competitive substrates, and that degradation of insulin-I^{131}, instead of demonstrating the direct removal of iodine, is a reflection of the degradation of insulin. Upon incubation of insulin-I^{131} and native insulin with a liver preparation, the rise in supernatant radioactivity has been related to the rise in nonprotein nitrogen. In addition, the rise in supernatant radioactivity has been related to the loss in biologic activity of insulin. Aside from indicating that insulin-I^{131} is a substrate suitable for studying insulin degradation in vitro, these studies suggested that in the liver, inactivation of insulin involves a proteolytic enzyme system. Proteolytic inactivation was also suggested by results of nonradioactive experiments performed with a partially purified preparation from liver. In this study, insulin was inactivated with concomitant rise in nitrogen and ninhydrin-reacting material in the TCA-soluble fraction. Using the perfused rat liver, Mortimore and co-workers found that the intact liver, as well, is capable of capturing and degrading a significant amount of insulin-I^{131}.

Although the number, nature, and mode of action of the enzyme or enzymes involved in the degradation of insulin have not been established, studies on inhibitors of insulin-I^{131} degradation have been performed with crude liver preparations. These experiments were carried out with the hope of finding a suitable agent that would be useful in the treatment of diabetes. While many compounds are inhibitory in the insulin-I^{131} assay, those more promising with respect to their hypoglycemic effects include l-tryptophane, plant growth regulators, and "insulinase inhibitor."

The above-mentioned uncertainties concerning the enzyme or enzymes involved in the degradation of insulin preclude definite conclusions concerning the substrate specificity of this unpurified system. How ever, it would appear that the system in question has some degree of specificity since a variety of experiments has indicated that several

native and iodinated proteins are not degraded. In addition, competitive effects of α-corticotropin, α-casein, glucagon, and growth hormone on insulin-I^{131} degradation have been noted. The following are some of the possibilities that might conceivably explain this phenomenon. Such compounds, not necessarily acting in the same manner, may be substrates for the same system, inhibitors of the same system, or degraded to inhibitory peptides by other proteolytic enzymes. Regardless of the uncertainties concerning the exact manner in which the degradation of various peptide and protein hormones is related, recognition of the relationship between these hormones around a degradative axis has proved of value. Such has been the case in interpreting data of in vitro studies in which the effect of one of these hormones has been measured in the presence of another.

Since many of the peptide and protein hormones besides insulin have direct or indirect effects on carbohydrate metabolism, better understanding of their degradative processes is also desirable. Several recent reports suggest that insulin may be a substrate for the same rat liver system that attacks glucogan. On the other hand, on the basis of differences between inhibitors of the insulin-inactivating and glucagon-inactivating systems of rat liver, Kenny suggests it is unlikely that the two systems are identical. Weisenfeld and co-workers report that insulin inactivation occurs much slower than glucagon inactivation does during perfusion through an isolated frog liver. The authors suggest that the enzymes which inactivate these two hormones are different, or, if identical, act at different rates. Mirsky and Perisutti found that aging, dialysis, or presence of citrate ions affects the ability of rat liver extract to degrade insulin-I^{131} without affecting its ability to degrade glucagon-I^{131} and other I^{131}-labeled peptides and proteins. On this basis they propose that the insulin-degrading enzyme is different from the others. Obviously, definitive studies of substrate specificity must await isolation of the inactivating factor or factors.

Because of the evidence cited above, the proteolytic process must be included when considering both the manner in which insulin is inactivated by liver and the substrate specificity of this inactivating system. It should be noted, however, that proteolysis of insulin by liver has not been definitively demonstrated. Assuming that proteolysis of insulin does occur in the normal course of inactivation by liver, the question arises as to the number of proteolytic enzymes involved. Applying the same assumption, it would also be of importance to compare the proteolytic enzyme or enzymes in question with cathepsins, which are currently being characterized by Fruton and his associates.

Although the splitting of peptide bonds is likely in the inactivation of

insulin by liver, inactivation by cleavage of disulfide bonds must also be considered. The liver contains sulfhydryl compounds such as cysteine and glutathione, which can inactivate insulin by reductive cleavage of disulfide bonds of insulin. The presence of such a heat-stable insulin-degrading factor in liver has been reported. Ultracentrifugal studies of Miller and Andersson suggest that in the reduction of insulin by thioglycollate (another sulfhydryl compound) there occurs, in addition to aggregation of products, formation of an appreciable amount of fragment or fragments much smaller than the original insulin. A study by Narahara and co-workers indicates that glutathione can cause TCA-solubilization of radioactivity from insulin-I^{131}. Paper electrophoretic experiments suggest that this radioactivity is not iodide. In addition to chemical reduction of insulin, enzymatic enhancement of reduction may also occur. Evidence for the presence of such a reductive enzyme in rat liver was recently presented by Narahara and Williams.

Although proteolytic degradation of intact insulin is possible, as has been demonstrated with pancreatic enzymes, reductive cleavage may increase the susceptibility of insulin to proteolysis. Of relevance is the report of Hill and Smith, who found that disruption of disulfide bonds of insulin considerably increases the susceptibility of insulin to the action of an aminopeptidase. If a reductive enzyme is isolated, its effect on other disulfide-containing hormones, such as prolactin, growth hormone, oxytocin, and vasopressin, would be of interest.

Insulin-I^{131} has proved very useful for following the purification by Tomizawa and Halsey of an enzyme that facilitates the degradation of insulin. It is extremely doubtful that this isolation of an apparently homogenous enzyme could have been achieved without an insulin-I^{131} assay. However, use of I^{131}-labeled peptides and proteins for enzyme purification purposes has certain limitations. Very important among these is the possibility of degradation in which all iodinated split products remain TCA-precipitable, resulting in undetected degradation. Another possibility is that degradation of a biologically active protein as determined by the I^{131}-labeled protein assay may not always be an indication of loss of biologic activity, since degradation of protein hormones without loss of biologic activity has often been demonstrated.

SUMMARY

The observations that insulin-I^{131} can be validly used under proper conditions in studying insulin metabolism is in itself noteworthy. By incorporating the use of insulin-I^{131}, significant progress has been made towards full understanding of the biological inactivation of insulin.

Demonstration of the presence of an acquired antibody in insulin-treated patients and the purification of an insulin-degrading enzyme using insulin-I^{131} are good in vivo and in vitro examples of situations in which this modified insulin has been most valuable. The degradation of insulin-I^{131} with appropriate control experiments has indicated that this is both in vivo and in vitro, an important and rapid process in the inactivation of native insulin. Prior evidence of degradative inactivation was much more indirect.

The liver quickly inactivates insulin by a process involving one or more enzymes. As to the enzymes, several possibilities exist as to their number and modes of action. A single proteinase or a reducing enzyme with absolute specificity for insulin might be involved. A single enzyme of either type with less than absolute specificity for insulin, as well as one or more proteinases and reductases each with its own substrate specificities, are also possibilities. Even less certain is the mode of inactivation of insulin by tissues other than liver.

The insulin-inactivating process may play a role in controlling the level of circulating insulin. However, this may simply be a means for removing hormone not used in exerting its biologic action. The present state of knowledge concerning the degradative process precludes evaluation of its role in the pathogenesis and course of diabetes mellitus.

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Chapter 22

GLUCAGON DEGRADATION

Hiromichi T. Narahara

The crystallization of glucagon and the elucidation of its chemical structure (Chap. 3) have lent impetus to the study of its metabolism. The investigations of Staub, Cox, Berson, and their colleagues, which suggested that glucagon could be labeled with I^{131} without appreciable loss of biologic activity, also facilitated such study.

Experiments with glucagon labeled with I^{131} (Chap. 6) have revealed that the compound rapidly leaves the blood stream of experimental animals; in adult rats, approximately 80 per cent of a dose of glucagon-I^{131} disappears from the circulation within 5 minutes after intravenous administration of the compound. This is accompanied by the appearance in the plasma of radioactive material not protein-bound, which is considered to represent the degradation products of the glucagon-I^{131}. Soon after the injection of glucagon-I^{131} into rabbits, radioactive material which is neither free iodide nor unaltered glucagon-I^{131} can be demonstrated in the plasma. Berson *et al.* have identified radioactively labeled monoiodotyrosine in this fraction. Eventually, this material also decreases in concentration within the plasma and is replaced by free iodide, which is ultimately excreted in the urine. In rats, practically none of the radioactive label appears in the urine or feces attached to

protein, thus, the major fate of glucagon appears to be degradation within the body.

Since plasma itself has little ability to degrade glucagon-I^{131}, the ratio of radioactivity not protein-bound to protein-bound in the plasma is a reflection of the rate of several concurrent processes: diffusion of glucagon into the tissues, degradation of glucagon in the tissues, diffusion of products of degradation back into the blood stream, and, finally, excretion of the degradation products and label from the body. Therefore, although the rate of accumulation of radioactive material not protein-bound within the plasma may serve as a rough index of the rate of degradation of glucagon-I^{131}, it cannot serve as a direct estimate of this rate. The rate of excretion of iodide in the urine may also be used as an indication of the rate of degradation, but there is a time lag between degradation of the protein and excretion of the label, and the possibility exists of reincorporation of label, particularly in the thyroid gland. Moreover, it has been reported by Yalow and Berson that radiation can alter labeled proteins and cause them to combine firmly with serum proteins, and this would affect the rate of metabolism of the altered moiety of the labeled compound.

The fact that protein-bound radioactivity (presumably largely glucagon-I^{131}) accumulates in the liver and kidney more rapidly than in other major organs or tissues after intravenous injection of glucagon-I^{131} into rats, and the observation that homogenates of these tissues are particularly effective in inactivating and degrading glucagon, suggest that these two organs are major sites of degradation of glucagon in the body. This concept is supported by the observation that removal of either the liver or kidneys from rats in acute experiments slows the rate at which glucagon-I^{131} is removed from the blood stream after injection. Although skeletal muscle accumulates less radioactive material than liver or kidney after the administration of glucagon-I^{131}, it possesses a moderately good capacity for degrading glucagon, and because of the large total mass of skeletal muscle in the body, this tissue should be considered as another important site of glucagon catabolism.

The inactivation of glucagon by an extract of rat liver has been shown by Kenny to be accompanied by the liberation of free amino acids from the protein. It has also been demonstrated in other experiments with glucagon-I^{131} that the production of radioactive material soluble in trichloroacetic acid (TCA) is paralleled by an increase in TCA-soluble protein derivatives that absorb light at 280 mμ. Berson and his colleagues have demonstrated by paper electrophoresis that free iodide represents only a small fraction of the products of degradation of glucagon-I^{131}, and chromatographic studies suggest that one of the products of degradation

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might be a labeled peptide. Thus, it appears likely that the biologic inactivation of glucagon and the formation of TCA-soluble material from glucagon-I^{131} in the presence of liver homogenates reflects cleavage of the peptide linkages of glucagon.

Vuylsteke and de Duve have noted that glucagon can be inactivated by a heat-labile system present in rabbit liver slices. Kenny has further characterized the glucagon-inactivating system of rabbit liver homogenates and has found that it is nondialyzable and is inhibited by p-chloromercuribenzoate. The enzyme system is localized in the soluble fraction of liver cytoplasm after sedimentation of the nuclei, mitochondria, and microsomes. These properties of the glucagon-inactivating system have been confirmed independently in studies on the degradation of glucagon-I^{131} by rat liver extracts, and it has also been shown that the enzyme system is optimally active at a pH of about 7.5.

It has been reported that the degradation of glucagon-I^{131} by rat liver extracts is impeded by the addition of either nonlabeled glucagon or insulin, under conditions where the rate of reaction is limited by the concentration of extract. Earlier studies on the inactivation of glucagon by Vuylsteke, Tyberghein, Kenny, and their collaborators had shown that the inactivation of glucagon by liver preparations in vitro could be inhibited by the addition of insulin, cysteine-inactivated insulin, the separated A and B chains of insulin, adrenocorticotropin, or growth hormone. Conversely, glucagon has also been found to decrease the rate of degradation of insulin by rat liver extract, and of corticotropic hormone by homogenates of rat anterior pituitary. The most likely explanation for the inhibition of glucagon inactivation by other proteins is that these proteins, or peptide derivatives of them, can act as competitive substrates for the proteolytic enzyme system which degrades glucagon.

This might suggest that insulin and glucagon are attacked by the same enzyme systems. However, certain differences have been noted between the degradation of glucagon and insulin by liver extracts. Mirsky *et al* have found, for example, that the degradation of insulin-I^{131}, but not of glucagon-I^{131}, is greatly decreased by dialysis of the extract or by allowing it to stand for 2 days at 5° C. It has been reported that incubation with glutathione can bring about an increase in the TCA solubility of insulin-I^{131}, but not of glucagon-I^{131}. If insulin differs from glucagon in that reductive as well as proteolytic processes enter into its degradation, then removal of glutathione from liver homogenates by autoxidation or dialysis might explain some of the phenomena that have been described. The finding of Kenny that iodoacetate and cupric ion inhibit the degradation of insulin by liver extracts more than they do the

inactivation of glucagon is also compatible with the view that these reagents might react more extensively with glutathione, which is necessary for the reductive degradation of insulin, than with proteolytic enzymes. It has been reported that the degradation of insulin-I^{131} by rat liver extracts is accomplished by a heat-stable system (which presumably includes reduced glutathione) as well as a heat-labile or enzymatic system, and that the heat-stable system is more readily inactivated by oxidized glutathione than is the heat-labile one.

The fact that insulin can spare glucagon from degradation of liver slices has been utilized to good advantage for assaying small quantities of glucagon in vitro.

It is interesting to note that the rate of disappearance of glucagon-I^{131} from the blood stream of rabbits has been found by Berson *et al.* to be unaffected by the administration of cortisone acetate in a dose as large as 50 mg. daily for 14 days. This would suggest that cortisone does not augment the activity of the proteolytic enzyme system which destroys glucagon.

In conclusion, the investigation of glucagon metabolism, which has been confined almost exclusively to experimental animals, indicates that it is rapidly inactivated in the body by proteolysis. The rapid degradation of glucagon may help to explain the short duration of its hyperglycemic action following the cessation of an intravenous infusion of the compound. Glucagon labeled with I^{131} is a substrate that can be used conveniently for the study of the behavior of a purified, small, rapidly metabolized protein in the body. One essential difference between the metabolism of glucagon and that of insulin is that the inactivation and degradation of glucagon appear to reflect a solely proteolytic process, whereas the catabolism of insulin is more complex and may be influenced by reductive as well as proteolytic processes.

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Chapter 23

EXPERIMENTAL DIABETES

Dwight J. Ingle

In open systems—each living thing is an open system—the same final state can be reached from different initial conditions and by different paths. This is the principle of equifinality, well illustrated by the many known ways of causing hyperglycemia and glycosuria in experimental animals.

The known methods of causing glycosuria by experiment are summarized in Table 23-1. Perhaps no one of them fully represents the cause of diabetes mellitus in man. Students of this subject are advised to read the book *Glycosuria and Diabetes* by Frederick M. Allen for orientation on the large number of facts known by 1913. Permanent diabetes can be induced in animals by a temporary disorder, such as by the administration of hormones or alloxan. Perhaps the cause or causes of clinical diabetes may also be inactive at the time the physician sees the patient.

There are important species differences and important sex differences in the responsiveness of experimental animals to diabetogenic stimuli and conditions.

Insulin is secreted by the β-cells of the pancreatic islets. The normal pancreas has the capacity to secrete more insulin than is required to

TABLE 23-1. METHODS OF CAUSING OR EXACERBATING EXPERIMENTAL GLYCOSURIA

I. Dietary
  A. Overfeeding
  B. Starvation
  C. Abrupt shift from high fat to high carbohydrate diet
II. Pancreatic (insulin) insufficiency
  A. Pancreatectomy
  B. Alloxan and related compounds
  C. Subtotal pancreatectomy and parenteral administration of high glucose load
  D. Parenteral administration of high glucose load to normal animals
  E. Subtotal pancreatectomy and prolonged injection of insulin.
  F. Abrupt withdrawal of insulin following prolonged injection
III. Hormonal
  A. Growth hormone
  B. Lactogenic hormone
  C. Corticotropin
  D. Adrenal steroids
    1. Hydrocortisone
    2. Cortisone
    3. Corticosterone
    4. 11-Dehydrocorticosterone
    5. Several synthetic derivatives of hydrocortisone
  E. 11-Oxygenated derivatives of progesterone
  F. Estrogens
  G. Thyroxin
  H. Epinephrine
  I. Pitressin
  J. Glucagon
IV. Stressors: acute response (glycogenolysis)
  A. Piqûre
  B. Emotion
  C. Asphyxia
  D. Trauma
  E. Toxins
  F. Drugs
V. Stressors: chronic response
  A. Infections
  B. Fractures
  C. Drugs
VI. Renal
  A. Phlorhizin and related compounds
  B. Uranium salts and other renal poisons

meet the ordinary exigencies of life. In this respect it is like all other organs concerned with the maintenance of homeostasis—it is endowed with a reserve that is not ordinarily tested to its limit. The metabolic signal to the release of insulin is thought to be the level of glucose in the blood that passes through the pancreas. Not only can the β-cells respond quickly to a change in need for insulin, but when a rat, rabbit,

or guinea pig is given a sustained high carbohydrate load the islets increase in size.

CARBOHYDRATE TOLERANCE IN NORMAL ANIMALS

ALIMENTARY GLYCOSURIA. The normal animal has the ability to assimilate much larger amounts of carbohydrate than are needed to meet energy requirements. A limited amount of the surplus is stored as glycogen and the remainder is converted to fat, a less bulky way of storing calories. However, the animal can be so glutted with carbohydrate that the assimilation limit is exceeded, the blood glucose rises, and glucose is excreted into the urine. Hofmeister observed in 1859 that overfeeding normal dogs with carbohydrate caused alimentary glycosuria.

Ingle has studied the capacity of normal rats to assimilate a high carbohydrate diet administered by stomach tube twice each day. When rats are tube-fed a normal caloric diet without prior adaptation, they may excrete glucose for a day or two, but following adaptation this does not occur again until excessive amounts of food are given. When normal rats are tube-fed a high carbohydrate diet at different rates of increment until death, very large amounts of diet are tolerated before the development of glycosuria. After the limit of tolerance is exceeded, each rat excretes significant amounts of glucose. The level of tolerance is independent of the rate of increment in food intake. The assimilation limit for the rat is at least twice the amount which such animals eat *ad libitum* to meet caloric needs. There is an important lesson in this observation. Any explanation of the metabolic defect in diabetes of either animals or patients must account for the loss of reserve tolerance that lies between the normal caloric intake and the assimilation limit as well as for the excretion of such amounts of glucose as are seen in diabetes.

When the normal rat is given an excess of carbohydrate the islets of the pancreas enlarge and contain more insulin. These changes are reversed to normal and the glycosuria disappears when the intake of food is decreased to normal.

Treatment of the overfed rat with insulin does not delay the onset of glycosuria, but it depresses the amount of urinary glucose below the level excreted by untreated rats and it permits the animals to tolerate larger amounts of carbohydrate before they are killed by overfeeding.

STARVATION DIABETES. In 1874, Lehmann observed that when dogs resumed eating following a prolonged fast, they excrete glucose into the urine. This is like vagabond's diabetes, described years ago in starving vagrants who were observed to excrete some sugar following hospitalization and refeeding. Ingle has reported on fasting diabetes in the

normal rat, which excretes glucose when tube-fed after fasting for ten days. Glycosuria can be induced in the starving rat by the force-feeding of a high carbohydrate diet representing less than normal caloric intake. The administration of insulin increases the carbohydrate tolerance of the starving rat only a little. Starvation diabetes is not caused by hypo-insulinism. There is abundant evidence that carbohydrate tolerance and utilization are affected by many factors.

In the tube-fed normal rat adapted to a high fat diet, a sudden shift to an isocaloric high carbohydrate diet causes a temporary glycosuria that can be only partially suppressed by treatment with insulin.

The carbohydrate tolerance of the normal animal and of man can be described as highly elastic: it adapts to wide changes in load, but normal elasticity can be reduced or destroyed in many nonspecific ways.

COMPENSATORY ATROPHY OF ISLETS. The continued administration of insulin causes the islets to become smaller and to contain less insulin. If, when the islets are resting, the administration of exogenous insulin is abruptly stopped, a temporary glycosuria may appear. A related clinical phenomenon is the temporary hyperglycemia that occurs following removal of islet-cell adenomas from patients with hyperinsulinism. It is probable that the secretion of insulin by the normal pancreas is suppressed by the excess released by the tumor and that, when the abnormal source of extra insulin is removed, the normal islet tissue remains hypoactive for a time.

PANCREATECTOMY

The classic method of producing experimental diabetes is to remove the pancreas, thereby creating insulin insufficiency.

PARTIAL PANCREATECTOMY. The amount of pancreas that must be removed in order to cause glycosuria depends upon the species and the dietary load of carbohydrate. In many animals on a stock diet *ad libitum*, 10 per cent or less of the total pancreas may suffice to keep the urine sugar-free. In rats, the islets of the pancreatic remnant increase in size and secretory activity as a compensatory response to the loss of insulin-secreting tissue. Little or no such visible compensation takes place in dogs and cats. In all species that have been studied, the assimilation limit for carbohydrate becomes less than normal; indeed, the removal of two thirds or even as little as one half of the pancreas limits the ability of the rat to cope with high carbohydrate loads. The partially pancreatectomized animal may also be more prone to develop diabetes following the administration of diabetogenic agents.

The secretory activity of a remnant of pancreas can be exhausted by

overwork in some species. The feeding of a high caloric diet to partially depancreatized, nondiabetic dogs can cause degenerative changes in the β-cells of the islets and ensuing diabetes. Similar exhaustion of the remaining β-cells occurs in the partially depancreatized cat given a high glucose load by parenteral administration. Dohan and Lukens produced diabetes in a normal cat by the same procedure. This cannot be accomplished in most normal cats or in other species. Even after extensive partial pancreatectomy of the rat, it has not been possible to cause irreversible damage to the remaining islets by overwork.

The experimental evidence on overwork of the β-cells in some species had led to the hypothesis that overwork of these insulin-secreting cells during periods of hyperglycemia can cause diabetes mellitus in man. The patient who inherits a pancreas of limited secretory capacity and then eats large amounts of carbohydrate may damage the β-cells and establish a vicious circle of events that lead to full-blown diabetes. The history of some patients is compatible with this hypothesis, but too little information is available to prove that the etiology of diabetes is this simple in any individual. Indeed, Ogilvie by histologic methods and Conn by a study of glucose tolerance give some evidence that the islets may be overactive in the obese prediabetic person. Most patients do not show extensive islet lesions analogous to those produced in partially depancreatized animals by overwork.

COMPLETE PANCREATECTOMY. The total removal of the pancreas rids the animal of the only known endogenous source of insulin, other possible pancreatic hormones such as glucagon, and (except in the teleost fish) the enzymes present in pancreatic juice. The possible role of glucagon in body economy is discussed in Chapter 15. In the absence of pancreatic juice the digestion of foods is not complete and some of it is excreted in the feces. The most obvious metabolic defects of the partially depancreatized animal concern the tolerance for and the utilization of carbohydrate. When the pancreas is completely removed the defects in metabolism of fats, proteins, and electrolytes become more clearly evident. The metabolic processes affected by insulin are described in Chapters 7 to 14.

Most studies on the results of pancreatectomy have been performed on the dog and cat, but in recent years the pancreas has been removed from the rat, rabbit, goat, pig, and fowl. Species differences in the consequences of insulin lack are well known. Mild diabetes, as measured by glycosuria, follows complete pancreatectomy in the herbivorous goat and duck, but is severe in the carnivorous dog and cat. It is interesting and important to note that pancreatectomy causes a marked rise in nitrogen excretion in all species that have been studied. The rat be-

comes severely diabetic following nearly total pancreatectomy and requires treatment with insulin in order to survive the operation. Most animals having severe diabetes eat larger than normal amounts of food until they reach the terminal stages of this metabolic disease.

ALLOXAN DIABETES

Alloxan causes diabetes by selectively destroying the β-cells of the islets. The severity of diabetes following administration of alloxan is determined by the extent of β-cell destruction. It is possible to find a dose of alloxan that causes complete or almost complete destruction of the β-cells, and the diabetes becomes permanent. Doses of alloxan causing maximal diabetes may cause some degree of damage to other organs, especially the kidney. When the initial glycosuria is mild it may disappear with time owing to the compensatory response of undestroyed β-cells, although the animal remains more sensitive than normal to other diabetogenic agents.

Alloxan is a useful tool for two general reasons. First, it permits the production of diabetes in animals that are difficult to depancreatize. Second, it destroys the β-cells without damage to the other secretory cells of the pancreas or to the supply of digestive enzymes in pancreatic juice. It is claimed that removal of the pancreas from an animal having alloxan diabetes causes some decrease in glycosuria and a decrease in insulin requirement. This has been presented as evidence that some other internal secretion of the pancreas, possibly glucagon, is an antagonist of insulin. The role of glucagon in the economy of the body is discussed in Chapter 15. The effects of pancreatectomy upon alloxan diabetes may be due to the loss of pancreatic juice, resulting in poorer nutrition.

When alloxan is injected into normal animals there is a triphasic change in the level of blood glucose: (a) immediate hyperglycemia, probably representing hepatic glycogenolysis; (b) hypoglycemia, especially severe in the rabbit, probably due in major part to the release of insulin from the damaged islets, and (c) hyperglycemia caused by deficiency of insulin.

Some compounds such as uric acid, dialuric acid, dehydroascorbic acid, and dehydroisoascorbic acid, each chemically related to alloxan, also cause islet necrosis. Other substances unrelated to alloxan have also been found to damage the β-cells; among them are magnesium and certain derivatives of quinoline.

A number of compounds have been found to prevent the diabetogenic action of alloxan if administered immediately before it. Nicotinic acid,

pyridine-dicarboxylic acid, 2-phenyl-quinoline-4-carboxylic acid, 1,2-dimethyl-4-amino-5(D-L-ribityl-amino)-benzene, 3,4-diaminotoluene, orthophenylene-diamine, and sodium bisulfite are protective in rabbits. Glutathione, cysteine, methylene blue, and British Anti-Lewisite are reported to be effective in rats.

HORMONES AND DIABETES

Most hormones cause hyperglycemia and even glycosuria when given in excess under one set of conditions or another. There is no evidence that the hormone of the parathyroid glands affects the metabolism of carbohydrate, but this possibility has not been exhaustively tested.

Each hormone that affects the metabolism of carbohydrate will do so in the absence of any other hormone, but the extent of response to a hormone can be altered by a change in the amount of another hormone in the body. Each hormone can affect several parameters of metabolism, consequently the end results of hormone actions are complex and cannot yet be explained in terms of mechanisms.

Removal of either the anterior pituitary or the adrenal cortex causes amelioration of diabetes and increased insulin sensitivity. Thyroidectomy may cause some decrease in the severity of glycosuria. The eviscerate animal has not been found to show increased sensitivity to insulin following hypophysectomy or adrenalectomy, but further study of this problem is needed. Some metabolic effects of hormones can be demonstrated in the eviscerate rat. It is generally true that when any two diabetogenic hormones are given together the effects are additive.

GROWTH HORMONE. The diabetogenicity of hypophyseal growth hormone (GH) is easily demonstrated in the dog, either partially depancreatized or intact. The continued administration of GH can cause metahypophyseal diabetes in dogs owing to hydropic degeneration of the β-cells, presumably by "overwork." There is a period of time in which metahypophyseal diabetes caused by GH can be "cured" by treatment with insulin, but when hyperglycemia has been sustained for several weeks the damage to islets becomes irreversible.

Other species are more resistant to the diabetogenic action of GH. The rat develops glycosuria in response to GH only when its reserve tolerance for carbohydrate is decreased as by partial pancreatectomy, the overfeeding of carbohydrate, or administering GH with another diabetogenic agent, so that additive effects become overt. The islets of the rat cannot be irreversibly exhausted by overwork, hence metahypophyseal diabetes has not been produced in this species.

The actions of GH are more completely described in Chapter 17.

PROLACTIN. The diabetogenic action of prolactin is very much like that of GH. It has been imagined that the diabetogenic action of each hormone is due to contamination by a third principle, but there is little or no positive evidence that this is the case. The metabolic effects of prolactin are described in Chapter 18.

ADRENAL STEROIDS. The diabetogenicity of adrenal cortical hormones was first demonstrated in depancreatized animals in which the diabetes had been ameliorated by adrenalectomy. Replacement therapy with large doses of adrenal cortical extract restored the glycosuria to the preadrenalectomy level. Next it was shown that the glycosuria of partially depancreatized rats could be exacerbated by large doses of adrenal cortical extract, cortisone, or hydrocortisone. It was then discovered that a diabetic state could be induced in normal rats by overdosage with these and other steroids, and by large doses of corticotropin either injected very frequently or continuously.

Glycosuria caused by hypercorticalism is called steroid diabetes. Steroid diabetes can be induced in rats fed a low-carbohydrate diet, but cannot be demonstrated in the fasting animal. Steroid diabetes tends to be insulin-resistant.

The highest order of diabetogenicity is found in naturally occurring and synthetic steroids oxygenated at position 11 of the nucleus. Massive doses of an 11-desoxy steroid may also exacerbate the glycosuria of the partially depancreatized rat, but this has not been tested in animals that are also adrenalectomized. It is possible that some 11-oxygenation of an exogenous steroid occurs in the body.

The intact dog and cat are rather resistant to steroid diabetes, but they are susceptible to the diabetogenicity of growth hormone and prolactin. The situation is reversed in the rat and other rodents. Buse, Gunderson, and Lukens did induce steroid diabetes in 7 of 9 intact cats by the administration of large doses of 9α-fluorohydrocortisone. The β-cells of the islets showed hydropic degeneration. The only two animals given prolonged treatment showed islet lesions progressing to early atrophy, and residual impairment of carbohydrate metabolism was demonstrable after the administration of steroid was stopped. Steroid diabetes is due in part to the overproduction of carbohydrate from noncarbohydrate sources in the liver and to inhibition of one or more phases of carbohydrate utilization. It is not certain that the oxidation of carbohydrate is suppressed in steroid diabetes. Effects of excess or insufficient steroids upon glucose tolerance can be demonstrated in the eviscerate rat, but the changes are not dramatic. The metabolic processes have not been identified and the role of these extrahepatic steroid effects in the economy of the body are unclear.

Steroid diabetes can be caused by large amounts of corticotropin in

those species which readily develop overdosage effects from hydrocortisone and related compounds. In order to stimulate the adrenal cortices to secrete an excess of steroids it is necessary to have corticotropin continuously present in the blood which goes to the adrenal cortices. This can be achieved either by the use of slowly absorbed corticotropin or by their frequent or continuous injection.

Steroid diabetes has been recognized in patients with severe clinical hypercorticalism caused either by an adrenal cortical tumor or by adrenal cortical hyperplasia. It is rarely seen in patients whose hypercorticalism has been induced by treatment with excess adrenal steroids or corticotropin, although this may occur in patients having latent diabetes mellitus. Testing effects of steroids upon glucose tolerance has been used successfully as a means of detecting latent diabetes mellitus in man.

It has been supposed that the increased secretion of corticotropin and of hydrocortisone during exposure to severe stressors might precipitate diabetes in either animals or patients with a subnormal pancreatic reserve. This possibility has been examined in some detail by experiments but the results have been uniformly negative. There is no evidence that a true state of hypercorticalism results when animals are exposed to stressors. The increased secretion of glucocorticoids serves to meet an increased physiologic need for these hormones and homeostasis is supported, not disturbed.

The metabolic effects of adrenal steroids are discussed in detail in Chapter 16.

SEX HORMONES. The body of information on experimental diabetes includes evidence for strange, poorly understood phenomena. The diabetogenicity of estrogens in the rat is one example. Administration of large doses of either natural or synthetic estrogens to the intact force-fed rat can cause a temporary glycosuria in some of the animals. When the reserve of islet tissue is reduced either by partial pancreatectomy or by alloxan, the administration of estrogen can cause a severe glycosuria in rats. The diabetogenicity of estrogen is not manifest in the rat eating *ad libitum,* since the estrogen causes anorexia, which masks the effect on carbohydrate tolerance. The continued administration of estrogen is accompanied by islet hypertrophy and eventually the glycosuria disappears. Even the spontaneous glycosuria of an alloxanized or partially depancreatized rat may disappear as the result of long-continued administration of estrogen. Estrogen has been found to be diabetogenic in the ferret.

The administration of large doses of estrogen to some patients with diabetes mellitus may suppress the requirement for insulin.

Massive doses of androgenic compounds may cause temporary exacer-

causing temporary hyperglycemia and glycosuria was first demonstrated by Claude Bernard in 1849. Lesions in other areas of the brain may have similar effects.

EMOTIONAL EXCITATION. In 1878, Boehm and Hoffmann reported on "Fesselungs-diabetes" in animals bound to an animal holder. Emotional glycosuria was more extensively studied by Cannon and his associates.

OTHER STRESSORS. Among the stressors that cause hyperglycemia and glycosuria by glycogenolysis in the liver are hemorrhage, asphyxia, removal of the carotid body, and parathyroidectomy.

DRUGS

GLYCOGENOLYTIC AGENTS. Hormones that cause hepatic glycogenolysis when given in pharmacologic dosages have been described above. The intravenous injection of salts may cause "salt-glycosuria." Almost any noxious or toxic or convulsive agent will, in certain doses, cause hepatic glycogenolysis.

ETHYLENEDIAMINE. Nonspecific stressors do not cause exacerbation of experimental diabetes. One corrosive liquid, ethylenediamine, does cause exacerbation of the disease in the partially depancreatized rat. When the injections of ethylenediamine are stopped the glycosuria falls to the preinjection level. The mechanism of action is known to be extra-adrenal, but is otherwise not understood.

SODIUM FLUOROACETATE. This drug, SFA, is converted to fluorocitrate, which inhibits the enzyme aconitase, hence blocking the tricarboxylic acid cycle. As a consequence of the accumulation of citrate and possibly of an accompanying suppression of insulin secretion, the rat poisoned with SFA develops a marked hyperglycemia with ketonemia, "SFA diabetes." This is an acute metabolic upset but it has not developed into permanent diabetes since the animal does not survive the continued administration of SFA.

RENAL POISONS

Phlorhizin and related compounds, uranium salts, and other renal poisons can cause a lowering of the renal threshold for the excretion of glucose so that glycosuria results without an accompanying hyperglycemia. Phlorhizin, discovered by Minkowski in 1893, has been an important tool in the study of metabolism, but phlorhizin diabetes is a treacherous state of abnormal physiology in which most of the parameters determining the endogenous production and metabolism of carbohydrate are unknown.

Lukens, Dohan, and Wolcott used phlorhizin diabetes as a device to lower the blood glucose of rats having pituitary diabetes. When this

was done within the first three months there was morphologic restoration of the islets and functional recovery *from the diabetes, thereby* supporting the hypothesis that hydropic degeneration of the β-cells is caused by overwork elicited by high levels of blood glucose.

COMMENT

There is abundant evidence for species differences in the response of animals to diabetogenic agents, and for genetic factors determining the susceptibility of the individual to these stimuli. The cause or causes of diabetes mellitus in patients remains unknown, but research on animals has given important new insights into the nature of this disease and how it should be treated. Actually, most of the methods for causing experimental *diabetes have been tested on man, either by nature herself* or by cautious clinical investigations.

Experimentation has not yet thrown much light on the relationship between insulin deficiency and the diseases of the vascular tree that appear in many patients with diabetes mellitus. There is an important time difference in the duration of experimental and clinical diabetes. Only a few depancreatized animals have been observed for as long as four years, whereas it usually requires diabetic patients a much longer time to develop arteriosclerosis even when their diabetes has been poorly controlled. Do animals with experimental diabetes develop humanlike degenerative changes? This possibility has not been adequately tested. Time is an essential commodity for the researcher.

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Chapter 24

PATHOLOGIC ANATOMY OF THE PANCREAS IN DIABETES MELLITUS

Philip M. LeCompte

HISTORY

It is perhaps fair to say that two major factors have influenced thinking about the pancreas in relation to diabetes in man: animal experimentation and inadequate histologic technique. The former has led to the sometimes unwarranted transfer of results in various animal species to man, while the latter has given rise to the widespread but false impression that the islands of Langerhans are "normal" in most cases of diabetes.

Three fairly distinct historic phases can be discerned.* In the first, which may be called the Weichselbaum era and occurred around the turn of the century, there was intense interest in the islands of Langerhans because of the careful anatomic studies of Laguesse and the demonstration that diabetes could be produced by removal of the pan-

* Extensive references cannot be given in a chapter of this size. The literature up to 1929 is thoroughly covered and admirably discussed in the monumental work of Kraus. The next most important review is that of Gomori. More recent papers, as well as older ones, are referred to by Ferner, Warren and LeCompte, and Gepts. An excellent recent survey is that of Seifert.

creas. The second phase, which has held sway until very recent years and is still of major importance, may be called the extrapancreatic era. It has been dominated by the discovery that hormones of other endocrine glands would produce a diabetic state when administered to animals in large quantities, and by the simultaneous realization that about half of all human cases, when examined by routine histologic methods, showed no definite lesion of the islets. The third phase, which may be called the quantitative era, began with the use of silver stains by Ferner and has culminated in the recent quantitative studies of Mac-

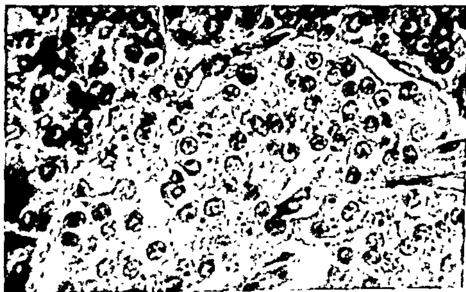


FIG. 24-1. Approximately half of a normal islet of Langerhans. For comparison with subsequent figures. Hematoxylin and eosin stain.

lean and Ogilvie and of Gepts, using the modern granule stains developed by Gomori.

LESIONS OF THE PANCREAS ASSOCIATED WITH DIABETES MELLITUS

The changes that may be found in the pancreas in human cases of diabetes may be conveniently considered under three headings: (1) gross destruction of pancreatic tissue, both acinar and insular, (2) "qualitative" changes in the islets, demonstrable with ordinary methods, and (3) quantitative alterations that can be detected only by the use of modern granule stains and estimation of total mass of β-cells and α-cells.

Gross Destruction of Pancreatic Tissue

PANCREATITIS. Transitory alterations of carbohydrate metabolism are, of course, common in acute pancreatitis, but usually disappear if recovery occurs. However, when cases of chronic pancreatitis are followed over a period of years a remarkably high percentage have been found to develop overt diabetes. Glucose tolerance tests have been found to be altered in a majority of cases of chronic pancreatitis in at least one series.

These findings are of interest not only because gross or microscopic evidence of fibrosis of the pancreas is a not infrequent finding in cases of human diabetes, but also because the mechanism of the presumed reduction in the supply of insulin is not clear. The question arises as to whether these cases are comparable to the diabetes produced by subtotal pancreatectomy in animals, where it is usually necessary to remove 90 per cent to 95 per cent of the pancreas in order to achieve a permanent diabetes. Unfortunately, accurate quantitative studies of the actual mass of islet tissue and of β-cells and α-cells have not been done in such cases (see Discussion at end of this chapter).

NEOPLASTIC DISEASE. Metastatic malignancy is, of course, most insignificant as a cause of diabetes. However, primary carcinoma of the pancreas is not uncommonly associated with the diabetic syndrome; not too rarely diabetes is the presenting symptom. In a series of 132 cases of cancer occurring with diabetes, 19, or 14 per cent, were primary in the pancreas (Warren and LeCompte). This may be compared with generally quoted figures of from 2.5 to 4.8 per cent for cancer of the pancreas in nondiabetics. In over half of these 19 cases the symptoms of cancer either antedated the symptoms of diabetes or occurred at about the same time, suggesting that destruction of the pancreas by tumor is an occasional cause of diabetes. In this instance also it is usually not clear how much pancreatic tissue is destroyed.

HEMOCHROMATOSIS. This is another disease usually considered to produce the diabetic state by destruction of β-cells. Such an assumption is, however, disputed by Bell, who doubts that the diabetes is attributable to damage to the β-cells, although others have been impressed by the remarkable predilection which the pigment seems to have for these cells. Here again is an instance where careful quantitative studies of the type to be described below might shed considerable light on the pathogenesis of diabetes in man.

SURGICAL REMOVAL OF THE PANCREAS. A few cases of total pancreatectomy that have been followed for several months are of interest in that their requirement for insulin is less than that of many spontaneous cases

of diabetes. However, the metabolic disturbance and the malnutrition consequent to the loss of the exocrine secretions of the pancreas are difficult to evaluate, and it is not justifiable to refer to these cases as "milder" than the spontaneous ones, especially since insulin requirement is only one method of evaluating the severity of diabetes (Lukens).

Qualitative Changes in the Islets of Langerhans

HYDROPIC CHANGE. This vacuolated or clear, empty appearance of the cytoplasm of the β-cells has been recognized for nearly sixty years. Its frequency according to various authors differs considerably, probably for two main reasons: (1) it is often difficult to distinguish from similar post-mortem changes, and (2) it can usually be clearly recognized and studied only in cases of recent onset in young people in whom autopsy is done very promptly after death. One criterion offered by Weichselbaum and by Kraus for the recognition of "true" hydropic change is the presence in the otherwise clear cytoplasm of "Kornchen," which are rodlike or droplike structures of uncertain origin staining bluish-grey or pinkish with hematoxylin and eosin (Fig. 24-2). These may be observed also in experimental material.

New light was thrown on the nature of this change when Toreson showed that glycogen could be demonstrated in the clear cytoplasm in some human and most experimental examples of it (Plate 24-1E). The failure of many other investigators to find glycogen in humans must certainly be largely due to the factors mentioned above, i.e., scarcity of suitable cases and lack of prompt fixation of tissue. The question is well discussed by Hartz and by Lazarus and Volk.

Much interest has been shown in hydropic change because it seems

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PLATE 24-1.¶*A.* Normal islet of Langerhans. β-Cells purple, α-cells red, Δ-cells green. Gomori's aldehyde-fuchsin, counterstained with his trichrome. For comparison with *B.* (Slide stained by Dr. G. Gomori.)

¶*B.* Juvenile diabetes, three-week history. Islet consisting of narrow ribbon-like cell cords, almost entirely α-cells (red granules). Lymphocytic infiltration ("insulitis") and increase of connective tissue. Gomori's trichrome. Compare with *A.*

¶*C.* Normal heavily granulated β-cells (would correlate with high insulin assay). Aldehyde-fuchsin, ponceau counterstain. For comparison with *D.*

¶*D.* Elderly diabetic. Part of islet consisting largely of α-cells (red granules) and a few sparsely granulated β-cells (low insulin assay would be expected). Same technique as *C.*

¶*E.* Hydropic change in human diabetes. Glycogen (red) in β-cells. Note absence of glycogen in adjacent duct. Periodic acid, Schiff reagent, counterstained with metanil yellow.

¶*F.* Margin of hyperplastic islet from infant of diabetic mother. Note giant nucleus and eosinophils surrounding edge of islet. Hematoxylin and eosin.

to be the earliest visible change in the islets in all types of experimental diabetes (except that caused by alloxan, and even here it may appear in surviving β-cells). For example, hydropic change appears early in experimental pituitary diabetes in the dog, and if the administration of pituitary extract is stopped at this stage the islets return to normal, i.e., the hydropic change is reversible. If, however, the extract is continued, the β-cells gradually disintegrate and the diabetes becomes permanent (see also Chap. 17). For this and other reasons, hydropic change has

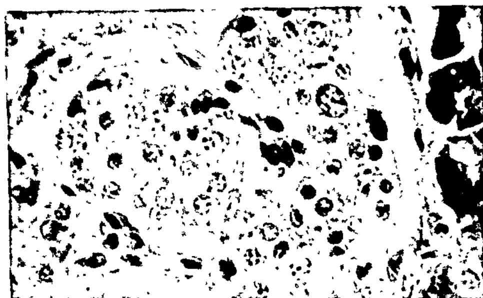


FIG. 24-2. Hydropic change with particles ("Körnchen") in cytoplasm of β-cells. Juvenile diabetes of recent onset. A giant nucleus is seen at right.

come to be regarded as a manifestation of "strain" or injury, the implication being that the β-cells are being called upon for an extraordinary output of insulin, as indeed seems likely from the experimental and clinical conditions under which this change becomes apparent. A transitory hydropic change can be produced simply by starving and then refeeding a rat, seemingly giving some confirmation to the strain hypothesis.

The proper interpretation of hydropic change is by no means obvious. Lazarus and Volk observed that, in rabbits given cortisone, glycogen appeared first in the ducts and later in the islets, and suggested that hydropic change does not necessarily indicate "strain" but may be another manifestation of the peculiar deposits of glycogen that occur in various tissues in diabetes (Warren and LeCompte). However, it

should be pointed out that glycogen is not necessarily found in the ducts in man when it is present in the β-cells (Plate 24-1E). It is of interest that hydropic change may be observed in spontaneous diabetes of animals (Fig. 24-3).

ATROPHY. It is not rare, especially in a diabetic of growth-onset type and many years' duration, to find extreme reduction or almost complete absence of islets. In addition to this apparent disappearance of insular tissue, the earlier authors described, chiefly in juvenile diabetics, islets composed of small "lymphocyte-like" cells, often arranged in cords or ribbons. This picture has been variously called "atrophy following hydropic degeneration" or "primary" or "simple" atrophy (Kraus) or even hyperplasia. Occasionally it is associated with infiltration with lymphocytes ("insulitis," see below). In recent years there is increasing evidence that these cords of small cells are composed almost entirely of α-cells (Figs. 24-4, 24-5, and Plate 24-1B), and for this reason they have been regarded by some as incomplete islands or "Teilinseln." Often there is an associated increase in the connective tissue of the islet. Not infrequently, along with the atrophy, one may find some evidence suggesting hyperplasia or regeneration in the same pancreas. Occasionally the narrow cords of α-cells may be found in the same islet with a few enlarged or hydropic β-cells.

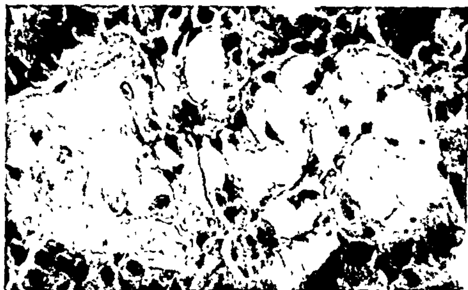


FIG. 24-3. Hydropic change (*right*) and hyaline (*left*) in same islet of spontaneously diabetic cat (Courtesy of Dr. Hans Meier.)

HYALINIZATION. This process, consisting of the deposit of a homo-

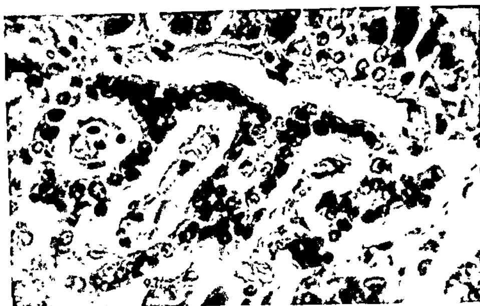


FIG. 24-4. Narrow cords of hyperchromatic cells ("atrophy") in juvenile diabetes of recent onset.

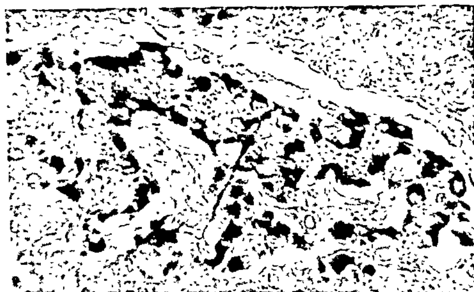


FIG. 24-5. Same case as Fig 24-1. Bodian stain. The cells are almost all silver-positive, hence probably α-cells. See also Plate 24-1B.

geneous, subendothelial, acidophilic substance, partially or completely replacing the islet, is the most obvious and therefore the most frequently recognized change in the diabetic pancreas. (Fig. 24-6) As a consequence, its importance may have been overemphasized (Bell). Certainly it seems to be related to age of the patient (most cases oc-

curring in older people) but not to duration or severity of the diabetes. There is no good evidence of any relation between the number of islets involved and the clinical picture of the diabetes. It has been stated, but not on incontrovertible evidence, that hyalinization of the islets may occur in nondiabetics.

The nature of the hyaline material is unclear. Several authors have reported that some cases give a positive stain for amyloid, but this throws little light on the chemical constitution of the substance. Some histochemical studies indicate the presence of acid mucopolysaccharides.

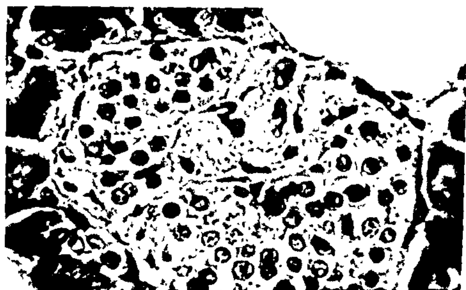


FIG. 24-6. Early hyalinization of islet of Langerhans (*upper center of field*).

Fat stains may show moderate to large amounts of fat in the hyaline as well as in the remaining β-cells (Fig. 24-7) but here again the pathogenetic significance of the observation is obscure. The theory that hyalinization of the islets is essentially a capillary sclerosis and hence the result of extension of arteriolar sclerosis appears to be unsupported by adequate evidence.

FIBROSIS. Minor degrees of interlobular and intralobular fibrosis of the pancreas, with more or less involvement of the islets, are common and extremely difficult to quantitate. Some authors have considered such evidence of "pancreatitis" as almost universal in cases of diabetes but they are certainly in the minority.

Increase in connective tissue with particular involvement of the islets is probably most common in juvenile diabetics showing the ribbonlike

islets variously regarded as representing atrophy or hyperplasia (Plate 24-1*B*). It may rarely be associated with lymphocytic infiltration, and in its early stage may appear as an apparent splitting of the capillary basement membrane, best visualized with special connective tissue stains.

LYMPHOCYTIC INFILTRATION ("INSULITIS"). The infiltration of the islets by small round cells, chiefly lymphocytes, is a lesion perhaps more important than its rarity would indicate. It is seen almost exclusively in



FIG. 24-7. Fat stain (Oil red O) of partially hyalinized islet of Langerhans. Fat appears black in photograph, and is seen as granules in islet cells at right as well as in mass of hyaline. Red cells outlining capillaries show subendothelial position of hyaline. (Courtesy of Mr. W. D. Wilson.)

young diabetics who come to autopsy within days or weeks after onset of the disease. Frequently the symptoms at onset may suggest an infectious process, but usually the circumstances are such that no search is made for mumps or other virus, and this possibility remains an unknown quantity. The cellular infiltration may be accompanied by hydropic change of the β-cells or, perhaps more often, by narrow, ribbon-like strands of cells usually identifiable as α-cells by the Gomori granule stains or, perhaps less reliably, by silver methods (Plate 24-1*B* and Fig. 24-5), although some authors find no granules in them and regard them as rudimentary cells (see above, Atrophy).

The interpretation of this interesting change is difficult. It is pre-

sumably important, since it appears to be associated with the initial injury or insult at onset of juvenile diabetes. It is evidently specific, since it occurs in the absence of involvement of the acinar tissue and may even occur simultaneously with an acute pancreatitis of different cellular type (polymorphonuclear leukocytes). It may represent actual invasion of the islets by an infectious agent or, perhaps more probably, a response to an unknown injurious agent. This agent presumably differs from the known methods of producing diabetes in animals, since such cellular infiltration is not known in experimental diabetes, although something like the cords of α-cells may be seen in alloxan diabetes in the rat.

NECROSIS. Selective necrosis of the islets is extremely rare and, when it does occur, is usually of obscure pathogenesis.

CALCIFICATION. This, too, is a rare curiosity, sometimes apparently superimposed on hyaline change, and of doubtful significance.

HYPERPLASIA AND REGENERATION. The ability or inability of the insulin-producing β-cells of the pancreas to reproduce themselves is probably a key factor in the pathogenesis of diabetes in man, as it certainly seems to be in experimental diabetes in various species of animals. Unfortunately, the evidence in man is often difficult to interpret.

Many observers have reported the presence of occasional large or "giant" islets in cases of diabetes, and Weichselbaum set an upper limit of 300 μ for the diameter of a normal islet. But mere size is not necessarily an indication of hypertrophy: for example, some of the large islets have been described as occurring in fibrotic areas and recently Eder has adduced evidence that the large islets found in the pancreas after duct ligation result, not from enlargement of a single islet but rather from the fusion or melting together of adjacent islets. The inference that hypertrophy of an islet has occurred may thus be illusory.

Various authors have described a ribbonlike pattern in the islets, interpreted as evidence of hyperplasia. Such a picture, in which the component cells have a columnar shape with the flat sides apposed to one another (Fig. 24-8), is not rare in the nondiabetic pancreas and may possibly indicate multiplication of cells, especially if they can be shown to be β-cells. (Sometimes they seem to contain no stainable granules of any kind.) However, in many cases of diabetes, especially in juveniles, the ribbons are made up of rather small cells with scanty cytoplasm, which, with modern granule stains, can often be shown to be α-cells (Figs. 24-4, 24-5) although in some instances they may not be identifiable (Gepts). This type of islet seems to have been regarded variously as evidence of atrophy (see above) or hyperplasia. It seems possible that it may arise from amitotic division of α-cells, as seems to

occur in the rat in alloxan diabetes; at any rate, it clearly does not represent regeneration in any effective sense but rather formation of an incomplete islet ("Teilinsel").

Another appearance that may suggest regeneration occurs when there is apparent continuity between acinar and insular tissue. Such a picture, which mimics stages in the embryonic development of the islets, is by no means uncommon in nondiabetics as well as diabetics, is sometimes associated with enlargement of nuclei of β-cells, and may indeed suggest hyperplasia, as in the case illustrated in Figure 24-9, where the

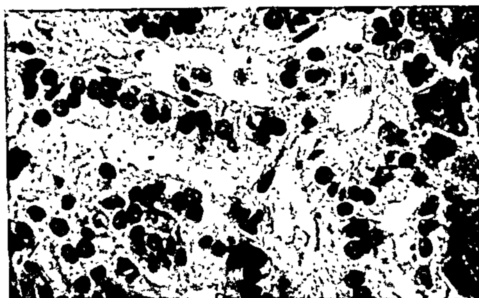


FIG. 24-8. Columnar or ribbonlike structure presumably representing hyperplasia. Nondiabetic. For comparison with Fig. 24-4.

patient was enormously obese and must have made heavy demands upon his insular tissue. On the other hand, a similar close relationship between acinar and insular tissue occurs in diabetes, frequently in connection with the ribbon-like pattern mentioned in the preceding paragraph, and in such cases cannot be convincingly interpreted as regenerative (Fig. 24-10). The whole question of whether acinar cells may be transformed into islet cells is still debated, the weight of opinion being apparently in the negative. (Kraus, Gomori, Hughes.)

The presence of ducts within or adjacent to islets may suggest hyperplasia but does not necessarily represent it, since small ducts and islets have a normally close relationship, and a duct may become enclosed in an islet during the developmental period.

Mitotic figures are extremely rare in the islets. It is noteworthy that

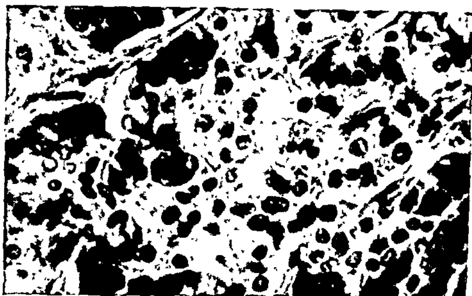


Fig. 24-9. Part of islet of enormously obese man, who had only recently become diabetic. A giant nucleus is seen at left center, and apparent continuity of islet and acinar cells at far left.

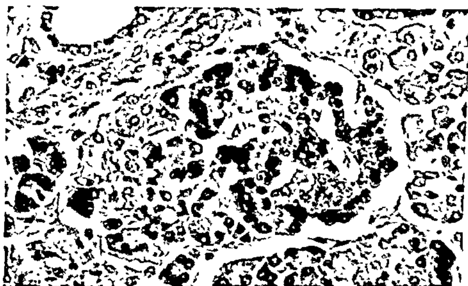


Fig 24-10. Islet of child diabetic of recent onset. At right are the narrow cords of small cells previously referred to (cf. Fig. 24-4), at left apparent continuity of islet with acinar tissue.

they seem to have been described more frequently in conditions such as hemochromatosis than in true idiopathic diabetes.

The size of nuclei in islet cells may have some validity as a criterion of activity (Rather). Enlarged nuclei (apparently usually, if not always, in β-cells) are seen frequently in nondiabetics, in the hyperplastic islets

of infants of diabetic mothers (Plate 24-1*F*), and in diabetics most often in the rare juvenile cases that come to autopsy soon after onset (Fig. 24-2). In such cases the enlarged, presumably hypertrophic β-cells may also show hydropic change and may be found in the same pancreas or even the same islet with the narrow cords of α-cells described above.

Gepts (1958) has described, in a few diabetics treated over a considerable period with oral sulfonylurea drugs, a peculiar type of hyperplasia characterized by swelling and proliferation of centro-acinar cells and multiplication of small ducts. The significance of this observation, as he points out, remains to be evaluated by further studies.

In conclusion, it seems clear that it is difficult to gauge the regenerative activity that may be going on at any given time in the pancreas. However, it is also obvious that whatever regeneration does take place within the diabetic pancreas is ultimately inadequate, since recovery hardly ever occurs and the insulin-producing tissue can be shown to be diminished (see next section below).

Quantitative Changes in the Islets of Langerhans

REDUCTION IN NUMBER AND SIZE. Some of the earlier writers were convinced that the diabetic pancreas contained fewer and perhaps smaller islets than the normal. However, this conclusion seems to have been often little more than an "impression," and when actual counts were attempted the methods used were not always above reproach, it being extremely difficult to arrive at an accurate estimate of the number of islets in a pancreas.

DEGRANULATION. A reduction in number of granules in the β-cells is common in diabetes and may be taken to indicate at least a temporary depletion of insulin in the pancreas, since it has been shown that the amount of β-cell granulation as revealed by the aldehyde-fuchsin stain usually correlates closely with the insulin content of the pancreas, although exceptions may occur (Wrenshall *et al.*). The stain just mentioned seems to be preferable to Gomori's earlier chrome-alum hematoxylin and phloxine method.* It must be emphasized that no conclusion at all can be drawn without the use of the proper technique. For instance, the islets in Plate 24-1*C* and *D* both looked "normal" with hematoxylin and eosin.

CHANGE IN β-α RATIO. A great stimulus to quantitative studies of the two major cell types in the islets was given by the work of Ferner, who applied the Gros-Schultze method of silver impregnation to frozen sec-

* The widespread use of the nonspecific phrase "Gomori's stain" is to be deplored, since the late Dr. Gomori not only devised at least two entirely new methods but also introduced valuable modifications of older techniques (see Chap. 4).

tions. (His conclusion that this stain is selective for α-cells is controversial, and its specificity is denied by many workers.) Ferner found the proportion of silver-positive cells to be increased in diabetes so that the ratio of β-cells to α-cells was reduced. In his earlier papers he regarded the silver-positive cells (α-cells plus a few others) as being the precursors of the β-cells and interpreted the altered ratio as a form of maturation arrest. More recently, since the discovery of glucagon, he has suggested that the reduced ratio of β-cells to α-cells represents a disturbance in the balance between insulin and glucagon and that this may account for some of the features of human diabetes. As noted elsewhere in this book, the hypothesis that glucagon plays an important role in human diabetes has not been substantiated. More important, perhaps, for present purposes, is that it now seems, from work to be quoted below, that the altered ratio is not due to an increase in α-cells but rather to a decrease in β-cells.

REDUCTION OF TOTAL MASS OF β-CELLS. Unquestionably the most important contribution to the pathology of diabetes in recent years has been the demonstration that *in practically all cases there is a reduction in the total mass of insulin-producing tissue,* i.e., the β-cells. Although this conclusion was hinted at in the work of Ferner about twenty years ago, and also to some extent by Gomori, it remained for Maclean and Ogilvie in Scotland and for Gepts in Belgium to carry out the studies necessary to establish it. These workers have, by painstaking methods, arrived at essentially the same final result, namely that, in practically all (a "quasi-totality," as Gepts puts it) diabetics there is a demonstrable reduction in the calculated total mass or weight of β-cells in the pancreas. This seems to occur without any definite alteration in the total mass of α-cells (thus accounting for the altered β:α ratio mentioned above). It is true that some overlap occurred with normal controls in both series, but this was slight and in general the differences were striking. For instance in Gepts' series, the average weight of β-cells in the diabetics was 0.301 Gm., compared with 0.754 Gm. for the nondiabetics, while in Maclean and Ogilvie's cases the corresponding figures were 0.218 and 0.641.

Maclean and Ogilvie were able to show that their results correlated well with those of Wrenshall *et al.* for extractable insulin in the pancreas, in that the most striking diminution of both weight of β-cells and insulin content occurred in the young diabetics (growth-onset type) which indeed also correlates with the results of studies of plasma insulin levels in the two major forms of diabetes.

Both groups of investigators relied chiefly on Gomori's chrome alum hematoxylin and phloxine method for their quantitative estimations, although Gepts also used a silver method for comparative studies. He, as

well as others, has found higher counts of α-cells with silver impregnation than with the chrome alum hematoxylin-phloxine technique, in spite of the fact that the latter does not distinguish Δ-cells, which are therefore necessarily included in the α-cell count. Results of this type have caused the specificity of the silver methods to be questioned and of late they have tended to fall into disrepute (see also Creutzfeldt).

SUMMARY AND DISCUSSION

It is customary for writers of textbooks of medicine or endocrinology to cite the supposedly "normal" islets of Langerhans in about half of all diabetics as evidence that insulin deficiency plays a minor part in many cases. It is evident from the work cited above that this is a misconception based on inadequate histologic technique, and that practically all cases of diabetes do indeed show a reduction of the mass of β-cells as compared with controls. This fact (which correlates well with findings on extractable insulin of the pancreas) strongly suggests that deficient secretion of insulin is of major importance in most cases of diabetes. This is not to say that other factors, such as insulin antagonists, may not also play a role. But the reduction in β-cells can hardly be a coincidence.

The question then arises as to how much insulin-producing tissue is "enough" to prevent diabetes. Our thinking in this respect has doubtless been clouded by the fact that it is necessary, in most animal species, to remove 90 per cent to 95 per cent of the pancreas to produce a lasting diabetes. However, the response to pancreatectomy does vary in different species, and the experimental results are not necessarily transferable to man. Certainly the quantitative studies cited above suggest that many nondiabetics are near the borderline as regards their β-cells, and that some unexpected extra burden or stress (e.g., the acquisition of obesity) might bring forth a latent diabetes. Such a supposition would seem to be supported by clinical experience. Furthermore, as noted above, in many of the cases of pancreatitis, cancer of the pancreas, or hemochromatosis, it would seem that only a moderate proportion of the β-cells are destroyed, and yet diabetes appears. This remains speculative until quantitative estimations of the β-cells are done; until this time, it would seem unnecessary to postulate either interference with the escape of insulin from the pancreas* or inactivation by "antagonists." In other words, it is conceivable that, as compared with most animals,

* It has been pointed out (Creutzfeldt, 1958) that such a blockade, or in other words a relative insensitivity of β-cells to the blood sugar level, could reasonably be postulated in maturity-onset diabetes, in which the β-cells seem able to respond to the sulfonylurea drugs by release of insulin.

man may have a high threshold level of available insulin, below which diabetes may be expected to appear. Whether excess glucagon may be involved, as postulated for the "obese-hyperglycemic" strain of mice (Wrenshall *et al.*) is uncertain.

Finally, if a reduced mass of β-cells is indeed a fundamental finding, the question arises as to how this comes about. Diabetic women frequently give birth to large babies who have hyperplastic islets of Langerhans. Some of these infants will develop diabetes in later life. Therefore it seems that islets which initially possess the power of regeneration may lose that ability in later life. One may say that some people have "ratlike" islets and others "doglike," but it also seems possible that the islets of diabetics may be peculiarly susceptible to some unknown injury that inhibits regeneration. That this injury may be a rapidly acting one is suggested by the manner of onset of juvenile diabetes and by the finding of Maclean and Ogilvie that the islets are of large size early in the course of the disease and later diminish rapidly in size and number.

Perhaps the newly discovered spontaneous diabetes of hamsters will throw light on the pathogenesis of human diabetes. In a strain of these animals there has appeared a spontaneous diabetes that is genetically transmitted, shows hydropic change followed by disappearance of β-cells, and is accompanied by renal and ocular changes that have not yet been fully studied (Meier and Yerganian).

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Chapter 25

HISTOCHEMISTRY AND ELECTRON MICROSCOPY OF THE PANCREATIC ISLETS

Paul E. Lacy

One of the ultimate goals of the pathologist in the field of diabetes is to correlate structural abnormalities of islet cells with their disordered function. In the past, the restricted resolution obtained by light microscopy has been one of the limitations that prevented a complete attainment of this objective. Now, with the advent of electron microscopy and new techniques in cytochemistry, fresh horizons are rapidly appearing that should remove the mantle of darkness from the intracytoplasmic organelles and their respective functions.

Recent technical advances in electron microscopy have made it possible to examine sections of intact tissue so that the nucleus, ergastoplasm, granules, mitochondria, Golgi complexes, and cytoplasmic membranes can be visualized simultaneously in one or several cells. The interrelationships of these cells and their association with capillaries and nerve fibers can also be demonstrated clearly. These technical advances are of such recent origin that only the initial steps have been accomplished on studies of the ultrastructure of islet cells under normal and abnormal conditions. Already cytologic features have been observed

that could not have been visualized or even predicted from light microscopic studies.

In the field of histochemistry, the fluorescent antibody technique developed by Coons and Kaplan has provided a tool that combines the specificity of an antigen-antibody reaction and the sensitivity inherent in the use of fluorescent material. This technique has been used for the demonstration of insulin in β-cells. Quantitative cytochemical methods are now available that utilize sections of cells as small as 100 $\mu \times$ 100 $\mu \times$ 20 μ (18), but the potentiality of these approaches for the study of islet cells has not yet been explored. Through the combined use of electron microscopy, histochemistry, light microscopy, and metabolic studies, a closer correlation should be achieved between structure and function of islet cells.

HISTOCHEMISTRY

PROTEINS. The first histochemical studies on islets of Langerhans were made by Lane, working in Professor R. R. Bensley's Laboratory. He found that granules of α-cells of guinea pigs were precipitated by 70 per cent alcohol and dissolved in aqueous chrome sublimate fixation: by contrast, β-cell granules were soluble in alcohol and precipitated by aqueous chrome sublimate fixation. Subsequent to these observations, several stains have been devised to demonstrate different types of cells in the islets. Lazarow has presented an excellent review of the development and modifications of these staining procedures. In most instances, the histochemical basis of the reaction involved is unknown. The staining of β-granules with aldehyde fuchsin is believed to be due to a reaction with sulfonic groups produced as a result of oxidation of cystine (24). Hartroft and Wrenshall have demonstrated that β-granules, stained with aldehyde fuchsin, represent insulin or an insulin precursor since a significant correlation could be made between the amount of insulin extracted from the pancreas of man and the number of β-granules observed in the cells.

A histochemical method for the detection of protein-bound *sulfhydryl* and *disulfide groups* has been developed and utilized for the demonstration of disulfide groups of insulin in β-cells (1). Recently, the fluorescent antibody technique has been used in order to obtain a more direct demonstration of insulin in islet cells (13). Antibodies to insulin are produced in the guinea pig, labeled with fluorescein isocyanate, and the resultant compound is used to demonstrate sites of its linkage with insulin within islet cells of frozen or frozen-dried sections of pancreas. A fluorescent reaction has been produced in the majority of the cells

within islets of bovine, hog, cat, dog, and rabbit pancreas. By indirect evidence the fluorescent cells in these species have been shown to be β-cells (Fig. 25-1). In the mouse and rat, a fluorescent reaction was ✓ produced in all the islet cells, whereas in the guinea pig and man a negative reaction was obtained. The latter findings suggest that an immunologic difference in endogenous insulin may exist in certain species, but further studies are needed to clarify these apparently anomalous findings.



FIG. 25-1. Demonstration of insulin in β-cells of normal dog pancreas by the fluorescent antibody technique. The fluorescent reaction in the β-cells was produced by staining a frozen-dried section of pancreas with labeled anti-insulin globulin. The pale cells near the center of the islet are α-cells which have a nonspecific blue autofluorescence. (× 350.)

Protein-bound *tryptophan* has been demonstrated histochemically in ✓ the α-cells of rabbit pancreas as well as in gelatin model slides of crystalline glucagon (17). A negative reaction was found in β-cells of rabbits. These findings suggest that the method demonstrates α-cell–glucagon since it has a high tryptophan content. However, the possibility exists that other α-cell–proteins containing tryptophan may contribute to their staining. If an antibody to glucagon could be produced, then the fluorescent antibody technique would again be of value in specifically demonstrating this substance within the cells. Initial studies on this problem have been undertaken in our laboratory but the results have been inconclusive thus far.

Hyalin is a homogeneous, eosinophilic substance containing an abnormal protein that is deposited in the islets of some diabetic patients. The exact nature of this protein is unknown but there is some evidence to indicate that it may differ from that in the hyalin commonly found in other structures, such as hyalinized glomeruli. In some instances it will stain metachromatically with methyl violet and resemble amyloid, while nonspecific hyalin does not give this reaction (28). Gamma-globulin has been demonstrated in amyloid by the fluorescent antibody technique so it will be of distinct interest to determine whether or not hyalinized islets contain this substance. Hyalin has also been observed to react as an acid mucopolysaccharide when it is stained with a colloidal iron technique. Hartroft stated that hyalin within some islets is often intermixed with large amounts of stainable lipid. In the routine examination of paraffin sections, one would not suspect the presence of lipid, although it is clearly evident in frozen sections stained with Oil Red O. The significance of the lipid in hyalin is not apparent at the present time but further studies may help to elucidate the relationship between these two substances as well as aid in determining the pathogenesis of hyalinization of the islets.

GLYCOGEN. Glycogen has been demonstrated histochemically in fetal pancreatic epithelium and in duct cells of normal, adult dogs (15) but it is not found in islet cells of normal animals. Toreson has shown that in experimental and clinical diabetes, glycogen is present in vacuolated β-cells. This vacuolation of β-cells had been considered previously as a type of hydropic degeneration, but with the work of Toreson it is apparent that the vacuoles represent an abnormal accumulation of glycogen in these cells. Lazarus and Volk have shown that glycogen accumulates in the ductular epithelium early in the development of experimental diabetes, whereas it becomes apparent in β-cells as a later phenomenon, depending upon the severity of the diabetes. They have considered these lesions as merely one facet of the generalized increased glycogen deposition occurring in the diabetic state and not as a degenerative process. Further cytologic and histochemical studies are needed to determine the functional status and ultimate fate of glycogen-infiltrated β-cells.

LIPIDS. Dogiel, in 1893, first described small fatty inclusions in the islets of Langerhans of man. Apparently, no significance is attached to this lesion with respect to human diabetes since severe degrees of lipid in the islets can be found in both diabetic and nondiabetic patients (28). By fluorescence microscopy, the islets of man are seen to contain varying amounts of brownish red-yellow fluorescent droplets (8). In many instances, the fluorescent islet can be clearly delineated from surround-

ing acinar tissue. This fluorescent material has been called "abnutzungs pigment," "wear-and-tear pigment," and *lipofuchsin*. It is indistinguishable from ceroid. This pigment apparently forms a part of the stainable lipid in the islets and may be partially derived from it. Large amounts of stainable lipid are also present in hyalin deposits of hyalinized islets as mentioned above.

HYDROPIC DEGENERATION. This condition refers to the presence of fluid-filled vacuoles in the cytoplasm of islet cells. Since a histochemical stain for fluid is not available, hydropic degeneration can only be established by excluding other substances (glycogen and lipid), which could also produce vacuoles. Experimentally, hydropic degeneration of α-cells can be produced by the administration of cobaltous chloride or synthalin. The vacuoles of these α-cells do not contain lipid or glycogen but they apparently do contain a small amount of protein in addition to the fluid since electron-dense material can be demonstrated within them by electron microscopy. As discussed previously, vacuolation of β-cells in diabetes is apparently always due to glycogen infiltration although it seems probable that a true hydropic degeneration of these cells might also occur under certain conditions.

ENZYMES. Relatively few histochemical studies have been made on enzyme systems of the islet cells. Gomori demonstrated *alkaline phosphatase* in peripheral islet cells of the rat and in the majority of islet cells of the dog, but it was absent from islets of other species. He was unable to obtain a positive identification of the cells, but in the rat the peripheral reactive cells corresponded to the usual sites of α-cells in this species. Fodden used longer periods of incubation and demonstrated alkaline phosphatase in all islet cells of the dog and cat, although the status of the reaction is uncertain under these conditions. The exact significance of the presence of alkaline phosphatase in islet cells is far from clear.

Tetrazolium salts are water-soluble compounds which undergo reduction to highly colored, water-insoluble substances in the presence of metabolic activity of living cells (2). Stier has demonstrated an increased reduction of tetrazolium salts in islets of guinea pigs within a few minutes after injecting glucose. The reduction was much greater in the islets than in surrounding excretory tissue, which suggests that glucose has a direct stimulating effect on the metabolism of islet cells.

METALS. Okamoto, in 1943, developed a histochemical method for the demonstration of zinc in islets. This method was based on the complexing of zinc ions with diphenylthiocarbizone (dithizone) or with its reduction product dithiozide to form a red-colored complex. Improvements in the specificity of this reaction have been made by utilizing

complex-forming buffer solutions that would mask its reaction with metals other than zinc. Since dithizone is also concentrated in islets when it is injected intravenously, this method has been used as an intravital stain for zinc (19). It has been demonstrated in the islets of several different species including man, but not in islets of the guinea pig. It is interesting that, in rabbits, dogs, and mice, zinc is found predominantly in β-cells, while in the rat a greater concentration is found in α-cells than in β-cells. Further discussion of the relationship between insulin and zinc will be found in Chapters 4A and 4B.

Hemosiderin has been demonstrated repeatedly in the islets of patients with hemochromatosis, but it was not until recently that the specific cellular localization of the pigment was determined (10). In hemochromatosis, almost all β-cells contain hemosiderin, but it is not observed in identifiable α-cells, which are greatly reduced in number in these cases. The significance of these provocative observations awaits the development of experimental methods for the reproduction of these changes in animals.

ELECTRON MICROSCOPY OF NORMAL ISLETS

IDENTIFICATION OF CELL TYPES. The various types of islet cells can be identified with light microscopy on the basis of differences in their tinctorial reactions. Since the islet cells can not be selectively stained before they are examined under the electron microscope, it was necessary to establish criteria for their identification. This objective has been accomplished by comparing the appearances of the same islet cells observed in low-power electron micrographs and in photomicrographs of contiguous sections stained with aldehyde fuchsin (11) (Figs. 25-2, 25-3). After repeating this procedure several times, distinct electron microscopic criteria were established for identification of different cell types. The same method has been used for the identification of α-cells and β-cells in the dog and rabbit. In the rat, the staining of the contiguous thick section was unsuccessful, so the α-cells were identified on the basis of their characteristic peripheral position in the islet of this species. This tentative identification has been verified by destroying the β-cells of the rat with alloxan and observing the affected cells by electron microscopy.

The general electron microscopic features applicable to both α-cells and β-cells are described below. The individual cells are enclosed by a distinct, continuous plasma membrane and their nuclei are surrounded by a double membrane. The plasma membranes of adjacent cells are closely applied in some areas while in others they are separated by

distinct intercellular spaces (Fig. 25-6). The cytoplasm of the α-cells and β-cells contains ergastoplasm, mitochondria, granules, and portions of the Golgi complex. The ergastoplasm is defined as the lamellar membranes with their associated small cytoplasmic granules (probably ribonucleic acid). The individual secretory granules (hormonal or prehormonal) are usually surrounded by a distinct, smooth membrane

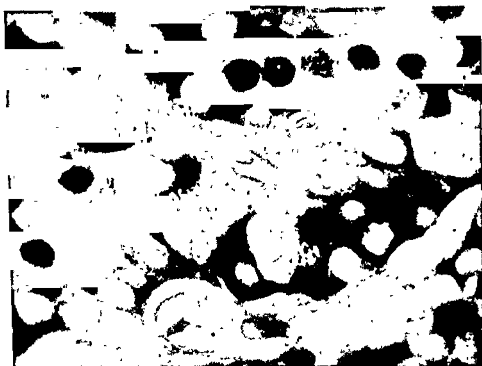


Fig. 25-2. Photomicrograph of a guinea pig islet stained with Gomori's aldehyde fuchsin method. The β-(black cytoplasm), α-(dark-gray cytoplasm), and C-cells (light-gray cytoplasm) adjacent to the capillary are also illustrated in an electron micrograph of a contiguous section (Fig. 25-3) (× 800.) (From *Anat. Rec. 128:* 255, 1957).

(Fig. 25-6). The smooth membranes comprising the Golgi complex are prominent and are observed in varying parts of the cytoplasm.

BETA CELLS. The ultrastructure of β-cells in the guinea pig, rabbit, rat, and dog is distinctly different in each of these species (12). These differences are so characteristic that the species can be identified on the basis of the electron microscopic appearance of their β-cells. In the guinea pig, the β-granules are scattered diffusely throughout the cytoplasm and they are round or irregularly shaped (Fig. 25-4). Occasionally clear spaces are present in the center of the granules. Their average

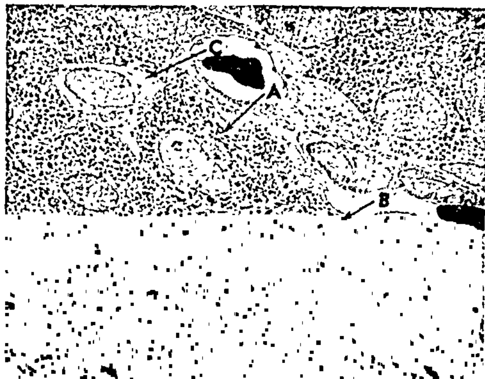


FIG. 25-3. Electron micrograph of the same islet cells illustrated in Fig. 25-2. The α-cells (*A*) have smaller granules, a lesser amount of *ergastoplasm and more ovoid nuceli than the β-cells (B). The C-*cells (*C*) have a pale cytoplasm and are devoid of distinct granules. (× 2,300.) (From *Anat. Rec. 128*:255, 1957.)

✓ TABLE 25-1. COMPARISON OF APPROXIMATE AVERAGE DIAMETER OF ALPHA AND BETA GRANULES IN DIFFERENT SPECIES

	Average diameter, mμ	
Species	*Alpha*	*Beta*
Guinea pig	180	230
Rabbit	200	130
Rat	180	170
Dog	170	. .

diameter is approximately 230 mμ, which is slightly larger than α-cell granules (180 mμ). A comparison of the approximate, average diameters of α-granules and β-granules in different species is shown in Table 25-1.

The distinguishing feature of β-cells in the rabbit is the presence of

a fine, fibrillar substance adjacent to the nuclei (Fig. 25-5). This fibrillar substance has not been found in islet cells of other species nor has it been described in other endocrine cells. It resembles the fibrils ✓ observed in glial fibers, muscle cells, and the tonofibrils of squamous epithelium. Egg albumin that has been incorporated into the cytoplasm of the yolk sac epithelium of the rabbit ovum also has a similar fibrillar

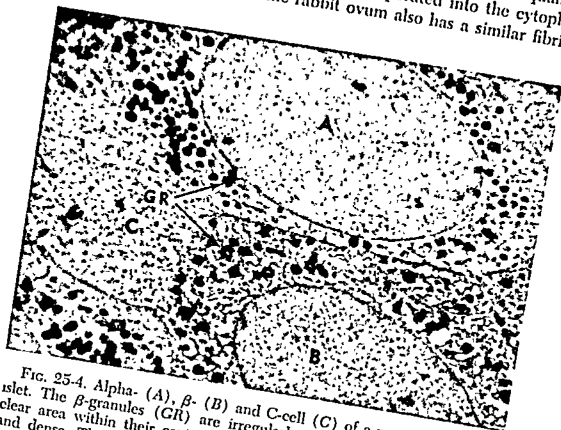


FIG. 25-4. Alpha- (*A*), β- (*B*) and C-cell (*C*) of a normal guinea pig islet. The β-granules (*GR*) are irregularly shaped and some contain a clear area within their centers. The α-granules (*GR*) are small, round, and dense. The β-cell (*B*) contains more ergastoplasm than the α-cell. The C-cell has a small amount of ergastoplasm and is devoid of distinct granules. (× 11,000) (From *Anat. Rec.* 128:255, 1957.)

appearance. The close resemblance of this fibrillar material to these various substances suggests that it may be a *sulfhydryl* protein. Further electron microscopic and histochemical studies may help to elucidate the composition and significance of this intriguing substance.

The difference between α-cells and β-cells in the rat is less marked in comparison with other species. The two types of cells can be distinguished, however, since the β-cells contain larger amounts of ergastoplasm and the secretory granules are less dense and are surrounded by clear spaces (Fig. 25-6). The granules of both cell types have round contours and are approximately the same size (Table 25-1).

The most striking electron microscopic feature of β-cells of the dog is the granules. The majority of the β-granules have a rectangular profile and each is found in a clear area bounded by a distinct membrane (Fig. 25-7). Occasionally, they appear club-shaped or assume the form of a V or T. The three-dimensional form of the granules is probably a disc or plate, since they usually extend completely across the sacs and very few empty sacs are abserved in the electron micrographs. The ex-

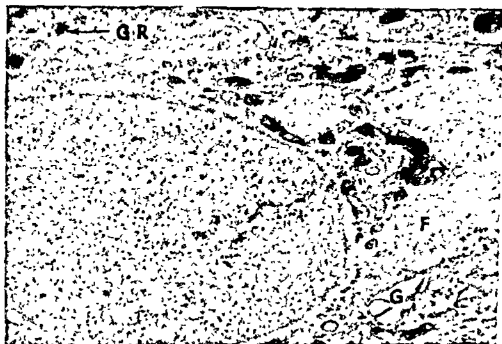


Fig. 25-5. A gray, fibrillar area (F) partially surrounds the nucleus of a normal β-cell of the rabbit. It extends irregularly into the cytoplasm between mitochondria (M) and the ergastoplasm. The β-granules (GR) are small and are surounded by a distinct membrane. G = Golgi complex ($\times$ 18,000.)

planation of this unusual appearance is unknown but possibly the rectangular configuration may be due to fluid accumulating in the sacs and compressing the granules into flattened discs or plates or they may represent a crystalline form of insulin or its precursors.*

* The β-granules also had rectangular profiles when the dog pancreas was fixed in either osmic acid buffered to different pH values or in 10 per cent Formalin. Rectangular granules were not found in other species even though the same methods of fixation, dehydration, and embedding were used. On the basis of these findings, it is apparent that the rectangular profile of the β-granules of the dog is not an artifact in the usual sense of the term.

ALPHA CELLS. In the species examined, the α-cells differ from β-cells in the following features: the concentration and density of α-granules are greater, the Golgi complex is smaller, and the amount of ergastoplasm is less than in β-cells (Fig. 23-4); α-cells have a relatively uniform appearance in each of the species.

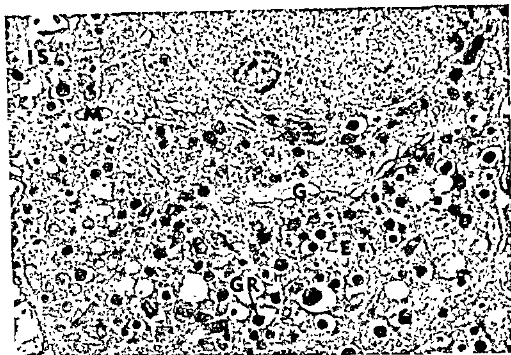


FIG. 25-6. The β-granules (*GR*) of a normal rat are round and are surrounded by a clear space with an outer limiting membrane. The smooth membranes of the Golgi complex (*G*) are present in several different areas of the cytoplasm. A large amount of ergastoplasm (*E*) is present between the mitochondria (*M*) and granules (*GR*). Small cytoplasmic projections of the β-cell extend into a distinct intercellular space (*IS*). (× 10,800.)

C-CELLS. The cytoplasm of C-cells contains only a small amount of ergastoplasm, scattered, indistinct mitochondria, a small Golgi complex, and no distinct secretory granules (Fig. 25-4). As with light microscopy, C-cells have been identified only in islets of the guinea pig. Further electron microscopic studies are needed to determine whether they represent a distinct type of cell or are simply degranulated α-cells or β-cells.

CAPILLARIES AND NERVE FIBERS. The endothelial cytoplasm forms a thin, continuous lining, although in focal areas it is markedly attenuated and its plasma membranes are so closely apposed that they appear as a

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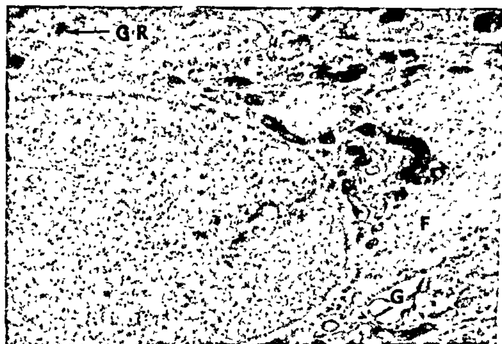


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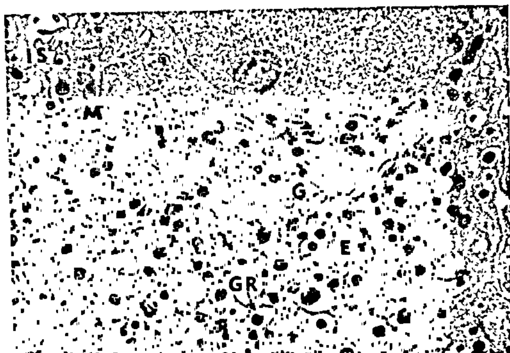


FIG. 25-6. The β-granules (*GR*) of a normal rat are round and are surrounded by a clear space with an outer limiting membrane. The smooth membranes of the Golgi complex (*G*) are present in several different areas of the cytoplasm. A large amount of ergastoplasm (*E*) is present between the mitochondria (*M*) and granules (*GR*). Small cytoplasmic projections of the β-cell extend into a distinct intercellular space (*IS*). (× 10,800.)

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FIG 25-7. A portion of a β-cell of the normal dog pancreas is illustrated. The granules (*GR*) have a rectangular profile and are surrounded by a clear space. Mitochondria (*M*) are scattered throughout the cytoplasm (× 20,000.) (From *Anat. Rec. 128*:255, 1957.)

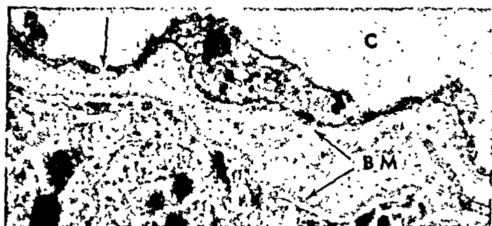


FIG. 25-8. Electron micrograph of a portion of a capillary (*C*) of a guinea pig islet. The plasma membranes of the endothelium are closely apposed in one area (*arrow*) and appear as a single line. Two basement membranes (*BM*) are present. One is associated with the endothelium and the other with the plasma membrane of the islet cells. (× 24,000.)

single line (Fig. 25-8). This type of endothelium is also found in other endocrine glands. Two basement membranes are present around the capillary (Fig. 25-8). One is a layer of amorphous material applied to the plasma membrane of the endothelium and the second is applied to plasma membranes of islet cells adjacent to the capillaries. These membranes do not extend into intercellular spaces. It is apparent that after secretory products leave the cell they must traverse two basement membranes and the endothelium before they can enter the capillary lumen.

Nerve fibers and their terminations on islet cells have been demonstrated by light microscopy. Occasionally, unmyelinated nerve fibers

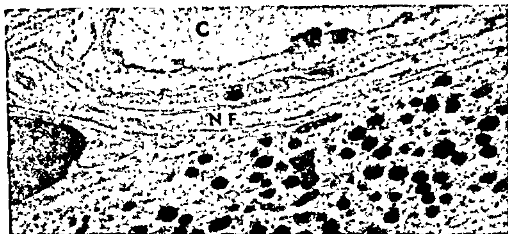


FIG. 25-9. Electron micrograph of an unmedullated nerve fiber (*NF*) in the islet of a hamster. The nerve fiber is surrounded by the cytoplasm of a Schwann cell and lies between the capillary (*C*) and the adjacent islet cells. (× 13,500.)

are observed in electron micrographs of islets of Langerhans (Fig. 25-9). Frequently, they are closely applied to plasma membranes of islet cells, but definite electron microscopic evidence of synaptic termination, sometimes referred to as neuroinsular complexes, on these cells has not been obtained as yet.

DEVELOPMENT OF ISLET CELLS. Munger has studied the cellular differentiation of the mouse pancreas from 13 days of gestation to 3 weeks postnatally. He found that islets develop from undifferentiated cells of the pancreatic anlage, from cells of ducts of the acinar pancreas, and possibly from centroacinar cells. All three types of precursor cells have similar cytoplasmic structure, with the granular component of the ergastoplasm predominating, an inconspicuous Golgi apparatus, and mitochondria with round to oval profiles. With the onset of cellular dif-



Fig. 25-10. Electron micrograph of a dilated intercellular space in the islet of a rat treated with large amounts of glucagon for 2 days. Long cytoplasmic processes (*CP*) extend from the β-cells into this dilated space. *N* designates nucleus. (× 12,000.)

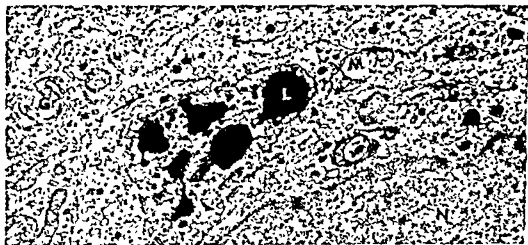


Fig. 25-11. β-cell from a 15-day-old embryonic mouse pancreas. Small β-granules as indicated by the arrow are diffusely scattered throughout the cytoplasm. The Golgi apparatus (*G*) is prominent and is composed of small agranular membranous sacs arranged in a parallel or circular fashion. Mitochondria (*M*) vary widely in shape, some being oval, others almost circular. Dense irregular lipoidal bodies (*L*) can be seen in occasional cells. The ergastoplasmic sacs (*E*) are not prominent. The nucleus of one cell (*N*) is located in the lower right hand corner of the photograph. (× 14,600.)

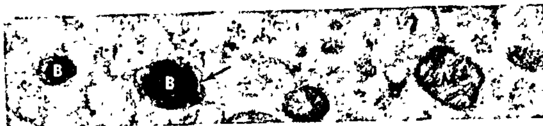


FIG. 25-12. Beta cell from a 15-day-old embryonic mouse pancreas. The β-granules (*B*) are seen in higher magnification and demonstrate the central dense portion of the granule, the surrounding clear area, and the outer limiting membrane (*arrow*). A small mitochondrion is seen to the right (*M*). (× 54,000.)

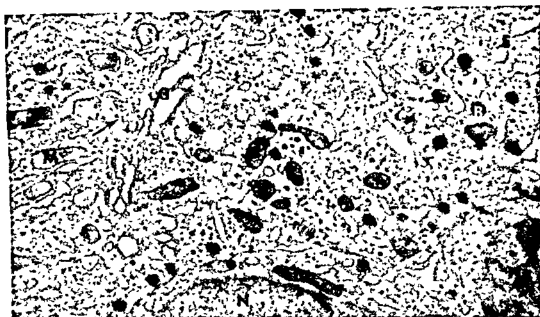


FIG. 25-13. Beta cell from an 18-day-old embryonic mouse pancreas. The cytoplasm is comparatively filled with organelles as compared with the 15-day-old embryo. The number of β-granules (*B*) is less than seen in the adult. The Golgi apparatus is very prominent and as indicated by the arrow is surrounded with numerous agranular vesicles that are thought to represent precursor stages of β-granules. The mitochondria are less varied in form than in the 15-day-old embryo. M = mitochondria; G = Golgi apparatus; N = nucleus; E = ergastoplasmic sacs. (× 17,000.)

ferentiation (demonstrated by the presence of specific β-granules), the membranous component of the ergastoplasm becomes evident with the formation of ergastoplasmic sacs (Figs. 25-11 and 25-12). The Golgi apparatus is more prominent, with numerous sacculations evident at its margins. The mitochondria at this time are rod-shaped with numerous parallel cristae.

In the mouse pancreas definite β-cells are seen by the fifteenth day of gestation. Many mature β-cells containing an abundance of ergastoplasm, distinct β-granules, and large Golgi complexes are present by the eighteenth day of gestation (Fig. 25-13). Immature β-cells persist until 2 weeks after birth. Between 2 and 5 days after birth, α-cells appear at the periphery of the islet and apparently develop from undifferentiated cells located in this position. There is apparently some species difference in the development of these cells since Nerenberg did not find α-cells with granules in the rat until 3 days after birth, whereas Bencosme observed mature α-cells in the developing pancreas of the rabbit at the twenty-sixth day of gestation.

These studies are in agreement with the opinion expressed by Ferreira, that β-granules are formed in the region of the Golgi apparatus by an accumulation of material in Golgi vesicles and gradual condensation of that material within the vesicle to form β-granules.

ELECTRON MICROSCOPY OF EXPERIMENTAL DIABETES

The application of electron microscopy to the study of experimental diabetes is possible, since the different types of islet cells can be recognized in the electron microscope and their general normal ultrastructure has been determined. The use of electron microscopy in this field should aid in determining the site of action of cytotoxic agents and the mechanism of formation, storage, and release of β-granules under conditions of physiologic and pharmacologic stress.

EFFECT OF ALLOXAN. The initial electron microscopic changes in islet cells of rabbits receiving diabetogenic doses of alloxan have been studied. A distinct increase in the number of β-granules is found 5 minutes after the intravenous injection of alloxan. It is not apparent whether this represents a stimulating effect of alloxan on β-cells or whether it inhibits the release of secretory granules. Regardless of the mechanism involved, the formation of β-granules in such an astoundingly short period of time suggests that their turnover rate may be very rapid under these conditions. Only remnants of the granules may be found in their sacs 15 to 30 minutes after the injection of alloxan (Fig. 25-14). Other definitive changes are not apparent until 2 hours after the injection. They consist of vacuolation of mitochondria, fragmentation of nuclear and plasma membranes, followed by a diffuse vacuolation and disintegration of the cell (Fig. 25-15). The necrotic debris is removed by macrophages, which are apparent after 12 to 24 hours. The specificity of the action of alloxan on β-cells is confirmed by electron microscopy since degenerative changes were not observed in α-cells.

EFFECT OF GLUCAGON. Temporary hyperglycemia and glycosuria have been induced in intact rats by the administration of large amounts of glucagon and by force-feeding the animals with a high carbohydrate diet (22). Electron microscopic studies have been made over a 7-day period on the pancreas of rats treated in such a manner. During the first 1 to 2 days of treatment, partial degranulation of β-cells occurred and

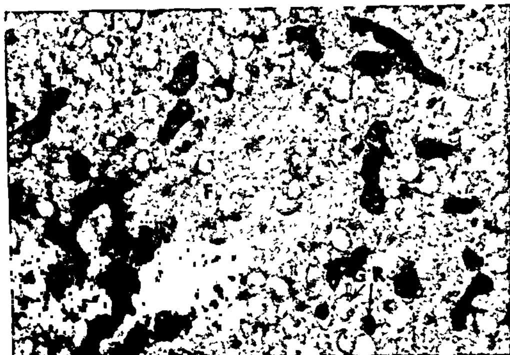


FIG. 25-14. Electron micrograph of a portion of a β-cell of the rabbit 30 minutes after injection of a diabetogenic dose of alloxan. An increase in the number of β-granules has occurred and only remnants of granules (*GR*) are present in the membranous sacs. Occasional distinct granules are present in some areas. The mitochondria (*M*) and fibrillar area (*F*) appear normal. (× 20,000.)

long cytoplasmic processes extended from these cells into dilated intercellular spaces (Fig. 25-10). Although their exact role in the process of secretion has not been determined, these elongated processes would obviously produce a tremendous increase in the surface area of the β-cells. With further treatment, the cytoplasmic processes and the intercellular spaces became less prominent even though an increased degree of granulation was present (Fig. 25-16). The mechanism by which insulin leaves the β-cell has not been definitely established. Apparently the β-granules undergo dissolution and their contents pass from the cells in a form that cannot be detected by either light or electron micros-

copy. Intact granules or portions of them have not been found in either the intercellular or pericapillary spaces, nor have they been observed within the cytoplasm of endothelial cells. The Golgi complex and mitochondria in degranulated cells appeared normal in size and distribution.

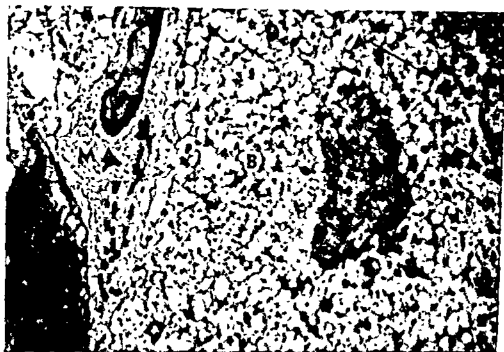


FIG. 25-15 Electron micrograph of a portion of a rabbit islet 12 hours after the injection of alloxan. The cytoplasm of the necrotic β-cell (*B*) consists of vacuolated debris and its nucleus is shrunken and pyknotic. The cytoplasmic and nuclear membranes have disintegrated completely. A macrophage (*MA*) is present in the necrotic debris. The ultrastructure of the α-cell (*A*) adjacent to the necrotic beta cell (*B*) appears normal. ($\times$ 12,000.)

EFFECT OF COBALTOUS CHLORIDE. Van Campenhout and Cornelis first produced vacuolation and degranulation of α-cells of guinea pigs by subcutaneous injections of cobaltous chloride. By electron microscopy we found the earliest changes in the pancreatic acini, not in the α-cells Partial degranulation, accumulation of lipid droplets, and degeneration of the mitochondria were present in pancreatic acini 24 hours after a single injection of cobalt, whereas changes in α-cells were not apparent until after 3 daily injections. The degenerative changes in acinar cells increased in severity with further treatment and giant mitochondria appeared with diameters more than twice normal. The large mitochon-

dria were called megamitochondria in order to distinguish them from the vacuolated forms commonly observed in degenerating cells.

The vacuoles produced in the α-cells were usually limited by a definite membrane and contained small amounts of gray amorphous material (Fig. 25-17). This substance was probably a protein diluted with fluid since histochemical stains for lipid and glycogen were negative. The cytoplasm between the vacuoles contained mitochondria, ergastoplasm,

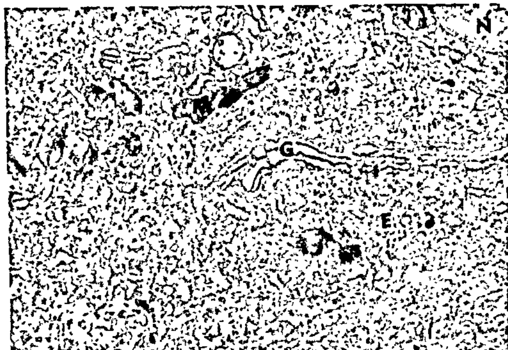


Fig. 25-16. Electron micrograph of a portion of a degranulated β-cell of a rat treated with large amounts of glucagon. The ergastoplasm (E) is abundant and slightly dilated in some areas. The mitochondria and Golgi apparatus (G) appear normal. M = mitochondria; N = nucleus. ($\times$ 22,500.)

secretory granules and portions of the Golgi complex. In some vacuolated cells, the mitochondria appeared dilated but in others they appeared normal even though large cytoplasmic vacuoles were present in the same cell. Because of this variability, the role of mitochondria in the production of the vacuoles could not be evaluated.

The vacuoles originated from ergastoplasm, since the membranes surrounding small vacuoles had cytoplasmic granules on their outer surfaces similar to normal ergastoplasm membranes (Fig. 25-17 *inset*). These ergastoplasmic sacs probably increased in size and fused to form large vacuoles. The vacuolation and partial degranulation were rever-

sible phenomena, for the ultrastructure of the α-cells returned to normal when the cobalt injections were stopped.

A direct effect of cobaltous chloride on α-cells was not established because of the long delay in the development of electron microscopic changes in α-cells. However, there was definite evidence of a direct action by cobalt on the acinar cells.

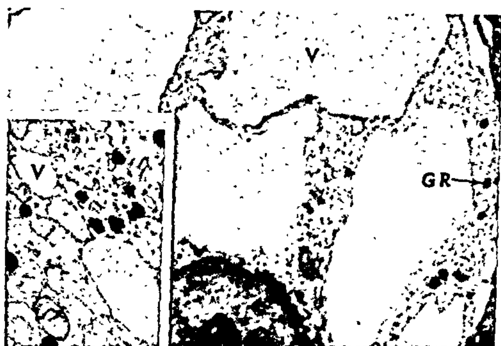


FIG. 25-17. Electron micrograph of a portion of a vacuolated α-cell of a guinea pig treated with cobaltous chloride for 4 days. The vacuoles (*V*) are limited by a membrane and contain a small amount of gray amorphous material. Alpha granules (*GR*), ergastoplasm, and normal mitochondria are present between the vacuoles. (× 12,000.)

(*Inset*) Small vacuoles (*V*) are illustrated in an α-cell of a guinea pig treated with cobaltous chloride for 3 days. The vacuoles (*V*) have cytoplasmic granules on their outer surfaces similar to the normal ergastoplasm. These vacuoles originate by dilation of the ergastoplasmic sacs and increase in size, forming the large vacuoles illustrated in the larger portion of this figure. (× 22,500.)

HUMAN DIABETES. At the present time, electron microscopic studies on the organelles of islet cells in human diabetes is limited, because it is necessary to use tissue that has been fixed within minutes after removal from its blood supply. This means that only biopsy specimens would be suitable for these studies. However, preliminary studies have indicated that it may be possible to utilize autopsy material for the

study of those structures which are not severely altered by autolysis, such as basement membranes, hyalin, and lipid within the islets. Since clinicians and surgeons are becoming more aware of the importance of electron microscopy in the study of disease, it appears very probable that in the future it will be possible to eliminate this deficient area in our knowledge of the ultrastructure of islet cells in human diabetes.

SUMMARY

A histochemical method is now available for the demonstration of insulin in the β-cell by the use of the fluorescent antibody technique. This method permits a detection of possible immunologic differences in endogenous insulin. Future adaptations of this procedure may make it possible to obtain quantitative estimates of the insulin present in the islet cells under conditions of physiologic and pharmacologic stress. Tryptophan has been demonstrated in α-cells of rabbits and on the basis of indirect evidence the demonstration of this substance may reflect the glucagon content of the α-cell. As yet, relatively few enzymatic studies on the islets of Langerhans have been accomplished.

The different types of islet cells can be identified and differentiated with electron microscopy in those animals which are commonly utilized in the study of experimental diabetes. The electron microscopic studies accomplished have unveiled details of the ultrastructure that could not have been visualized or even predicted by light microscopy, as exemplified by the rectangular outline of β-granules of the dog. Electron microscopy is also being used for the study of changes in the ultrastructure of the islet cells in experimental diabetes. The future adaptation of both the techniques of electron microscopy and cytochemistry should aid greatly in understanding the mechanisms of formation, storage, and release of the secretory products of the islet cells under normal conditions and in diabetes.

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Chapter 26

PATHOLOGIC ANATOMY FROM ABNORMAL INTRAVASCULAR FAT

W. Stanley Hartroft

Recently obtained evidence emphasizes anew the importance of disturbances of fat transport and metabolism in the diabetic state. The dramatic lowering of blood levels of nonesterified fatty acids by insulin, although a relatively new observation, is now firmly established (see Chap. 12). The frequency of hyperlipemia in untreated diabetics is a very old but equally relevant observation. The importance of insulin in promoting normal deposition of fat in adipose tissue could not have been demonstrated more strikingly than in the early photographs published by the Toronto group to illustrate the (then) astonishing transformation of cachetic juvenile diabetics into normal "roly-poly" children when insulin was administered to them early in the 1920's. There is now evidence suggesting that when insufficient insulin is available, fat not only fails to be deposited in normal storage sites in adipose tissue but also is abnormally deposited in vital areas, producing pathologic changes. Continual increase in knowledge concerning alterations of lipids in diabetes lends credence and import to observations of pathologists who have iterated the ubiquity of abnormal fat deposits in almost all anatomical lesions associated with diabetes and its complications.

Available biochemical and anatomical data suggest that abnormal deposition of fat deserves consideration as a possible fundamental, if not primary, pathogenic event in islet, renal, and retinal lesions of diabetes. The relation here to atheromatous fat deposits and resulting vascular complications is also indicated but cannot be included for lack of space. Even yet, the possibility has not been eliminated that islet lipoidosis may have significance in the development of some forms of the diabetes per se in adults and is not merely restricted to its later complications. In this chapter will be described lipid in lesions in man and relevant observations in animals.

ABNORMAL LIPID DEPOSITS IN ISLETS

Any significance attributable to abnormal deposition of fat in the islets of Langerhans in diabetes appears at present limited to that form of the disease developing in adults. In the juvenile type, islets are extremely few or absent. Beta-cells when present exhibit degranulation and atrophy but abnormal deposits of fat are rarely encountered. Amounts of extractable insulin in these pancreases are greatly reduced below normal, or cannot be detected at all (mouse bio-assay method), as might be expected from the anatomical findings.

In sharp contrast with this situation in the diabetic child, almost normal amounts of extractable insulin and a wide variety of islet changes are the rule in pancreases of adult diabetics examined at autopsy. Experimental studies have thrown little light on pathogenesis of these lesions in adults because morphologic counterparts do not develop in islets of animals in which diabetes has been produced by any one of several of the procedures now available (pancreatectomy; administration of alloxan, pituitary extracts, adrenal cortical hormones, or repeated intraperitoneal infusions of glucose). For this reason, most of the discussion to follow necessarily is based on autopsy studies. Production of a type of diabetes in animals comparable to the common form in adult man and in which the pancreases would contain almost normal amounts of insulin in hyalinized or fibrosed islets has not been achieved. Attainment of this goal will represent an advance difficult to overestimate.

The usual roster of islet abnormalities found in adult diabetics includes hyalinization, fibrosis, vacuolation of β-cells with glycogen deposits, hypertrophy or hyperplasia, atrophy and, in a small number, iron deposits in the special instance of hemochromatosis (see Chap. 24). Reduction in numbers of β-granules may or may not accompany the foregoing changes. In most cases, both granules and extractable insulin are abundantly present. In 15 to 25 per cent, pathologic changes

(either quantitative or qualitative) may be completely undetectable. Ubiquitous to any of the various lesions, when present, is an accompanying deposition of abnormal stainable lipid. In some 80 per cent of diabetics in whom islet lesions can be demonstrated, fat deposits are a prominent feature in suitable preparations. In our laboratory we have found abnormal lipid within β-cells, in or around the basement membranes in capillary walls and lumens, or even in all these sites in single islets. Lipid is regularly found in areas of islet hyalinization or

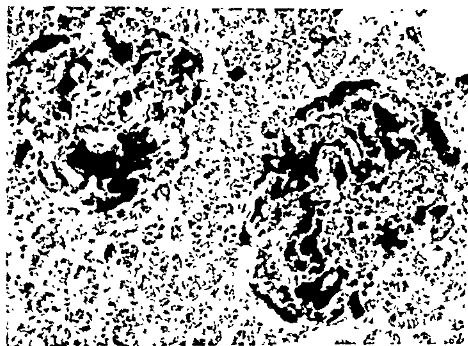


FIG. 26-1. Two islets, from the pancreas of an adult diabetic, are filled with masses of stainable lipid (black in photograph) that stand out clearly in this frozen section stained with Oil Red O.

fibrosis. Also, fat in walls of nutrient arterioles that supply islets is often abundant.

Abnormal lipid accumulates in more islets and in larger amounts in pancreases of adult diabetics than in those of comparable nondiabetics. Its presence in islets of some nondiabetic individuals has led pathologists to question the significance of its role in either the etiology or the pathogenesis of the disease. But neither can the changes of islet hyalinization and fibrosis be regarded as pathognomonic of diabetes because they too are found in nondiabetics. Despite this fact, greater attention has been paid to these other lesions than to islet lipoidosis. At least until recently, the consensus was that little significance could be attached to

the fat. We do not have data to refute this viewpoint but cannot agree that definitive evidence in support of the pathogenic significance of islet hyalinization and fibrosis is stronger than that for lipoidosis. Final evaluation must await development of methods for the production of all these changes in islets of animals, either diabetic or nondiabetic.

In least affected islets of diabetic patients examined at autopsy, stainable lipid may be restricted to relatively small intracellular droplets within cytoplasm of β-cells. But larger amounts, both within and without parenchyma, are sometimes so great that every islet in the Oil Red O-stained, frozen sections is clearly visible even under the scanning lens (Fig. 26-1). In hyalinized and fibrotic islets the amount of lipid is variable but sometimes relatively abundant. We suspect but cannot establish that the amount of fat may vary with stages of lesions, decreasing as fibrosis increases. Stainable lipid in affected islets may equal or exceed the amount of associated hyaline or fibrosis tissue. Particularly in hyalinized (rather than fibrotic) islets, fat is prominent and abundant although infrequently given the emphasis directed to the hyalin. The methods commonly employed are probably responsible for this disparity, because for every islet studied in sections prepared to preserve and demonstrate fat, literally hundreds and even thousands are examined in paraffin sections from which all fat is removed during their preparation. If the relative popularity of the two methods had been reversed in the past, islet hyalin would undoubtedly have been much less discussed because in frozen sections it is as effectively obscured by the brightly stained fat (Figs. 26-2A, 26-2B) as is the latter enhanced. Exactly the reverse situation exists, of course, in paraffin sections, because here only the hyalin can be seen. Hyalinization of nutrient arterioles supplying islets is also a frequent accompaniment of hyalin deposition in the islets and some have suggested that resulting ischemia may be responsible for the islet change. In these thickened arterioles again, lipid is quantitatively as important as hyalin (Fig. 26-3).

For these reasons and because "hyalinization" in other sites in diabetics (coronary arteries, kidneys, retinae) is accompanied by abundant pathologic deposits of lipid, we now refer to the process as *lipohyalinization* Reference to the deposits as *lipohyalin* emphasizes the abnormal lipid component. The significance of lipohyalin in islets is unknown but its presence suggests consideration of a hypothesis that lipid deposits, with or without hyalin, interposed between β-cell granules (usually present in abundant amounts) and capillary lumens may interfere at some point with passage of insulin from granules to blood stream in some adult diabetics. Methods of producing deposition of islet lipo-

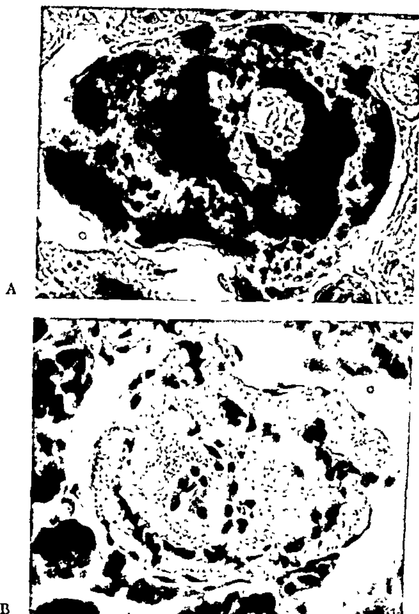


Fig. 26-2. These photomicrographs, taken at a higher magnification than the preceding one, demonstrate the relative quantities of fat and hyalin in this abnormal islet of an adult diabetic. (*Top*) A frozen section stained with Oil Red O, illustrating masses of abnormal lipid (black) deposited in and between the islet cells and capillary walls β-cells are atrophic with sparse granules and abnormally small, dark nuclei. (*Bottom*) The same section after all pathologic lipid was extracted with ethanol and xylol and the slide restained The hyalin (previously obscured by the fat) is now clearly apparent in the form of gray, somewhat granular masses. Quantitatively the fat and hyalin are approximately equal, justifying the term *lipohyalin* for this type of abnormal deposit.

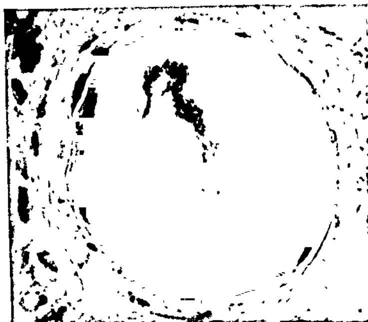


FIG. 26-3. A nutrient arteriole of an islet of an adult diabetic is illustrated in a frozen section stained with Oil Red O. Its lumen is practically occluded by masses of *lipohyalin*.

hyalin in animals are sorely needed for investigation of these possibilities; the means whereby to achieve this end are worthy of vigorous pursuit. Even if the hypothesis were disproved, a second possibility for further consideration would remain. Substances in the blood that stimulate release of insulin from β-granules (glucose, tolbutamide) might conceivably be considerably less effective when separated from the granules by barriers of fat.

PATHOLOGIC FAT IN KIDNEYS AND EYES OF DIABETICS

In the course of studying organs of rats that had been subjected to prolonged periods of dietary lipotropic deficiency, the writer unexpectedly encountered glomerular lesions that resembled closely the focal nodular form of diabetic glomerular sclerosis (Kimmelstiel-Wilson). In studying all stages of the glomerular changes present in the animals their pathogenesis emerged. In livers of these rats, large fatty cysts develop which subsequently may rupture (and in this way lead to scarring, fibrosis, and eventual cirrhosis), permitting escape of globular fat into the blood stream. The intermittently released fat emboli along with particles of ceroid (a special form of lipid formed in these livers) were found in vessels of lungs and kidneys. In the latter, fat emboli were found entrapped within glomerular capillaries

and the affected portion of the tuft sometimes appeared locally necrotic. The distribution and size of these acute focal changes corresponded to the chronic nodular lesions first encountered. Although these choline-deficient animals were not diabetic, the results indicated that fat emboli of nontraumatic origin could induce focal glomerular lesions resembling in many ways those associated with diabetes. Common to both conditions, choline deficiency and diabetes, is a disturbance of

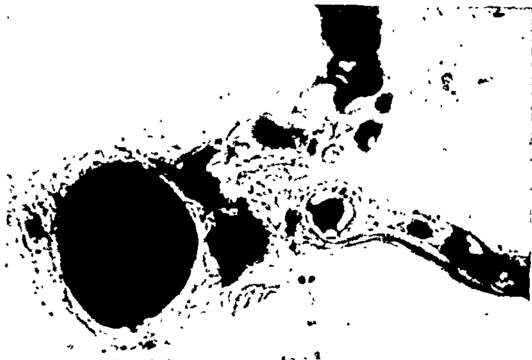


Fig. 26-4 A pulmonic alveolar arteriole and septal capillaries are completely filled with fat emboli (*black*) *in this frozen section stained* with Oil Red O prepared from the lung of a patient (nondiabetic) dying of fatty alcoholic cirrhosis. Similar fat emboli have been observed in lungs of diabetics (see text).

fat transport and metabolism. Kidneys of diabetics with Kimmelstiel-Wilson disease were therefore examined by the frozen-section technique to determine if there might be found here too any evidence of a similar type of pathogenesis. Support for this hypothesis was provided by results of other investigators (1, 2) (Fig. 26-4) who found that nontraumatic fat embolism of a chronic intermittent type could indeed exist in man in association with fatty livers in alcoholics. Later this finding was extended to diabetes (3) although the functional significance of the emboli was not established.

Almost a dozen reports of the glomerular lesions first described by Kimmelstiel and Wilson, including their own, make reference to the presence of abnormal stainable fat. Few attributed pathogenic significance to the fat, however, and none of the investigators had observed it in the form of intravascular plugs. In our published studies of kidneys of 16 diabetic patients in which Kimmelstiel-Wilson lesions were present, intraluminal fat plugs were clearly demonstrated within glomerular capillaries in 12, as well as within other preglomerular and postglomerular vessels. This percentage of positive findings has since been extended to and confirmed in a larger series. Fat emboli were found in only 3 of 22 diabetics without Kimmelstiel-Wilson lesions. In kidneys of 33 nondiabetic patients with miscellaneous renal lesions including glomerulonephritis and nephrosclerosis, fat was found within glomerular vessels in only 4, all of which were from patients with alcoholic fatty cirrhosis.

The intravascular fat in the glomeruli of the diabetics with Kimmelstiel-Wilson disease was remarkable for its solid pluglike form, its intense sudanophilia and, in some instances, a crystalline appearance in the sections (Fig. 26-5), whereas in the alcoholics the lipid was in much finer droplet form. In these glomeruli, abundant fat deposits were present not only within the capillaries, but also in their walls, between them, in the space of Bowman, in the glomerular capsule, and even precipitated on basement membranes of tubules.

In the choline-deficient rats the sequence of events leading to focal glomerular sclerosis was clearer than in the diabetics. Fat emboli in the animals had produced local obstruction of glomerular capillary tufts with subsequent degeneration accompanied by focal plasma exudates which become eventually converted into nodules of lipohyalin. The liver is almost certainly the source of fat emboli in these rats. In the diabetics, however, origins of the fat plugs in glomerular capillaries were not obvious. At death, fatty cysts in the liver were not found consistently enough to correlate with the presence of glomerular lesions. Similar plugs of fat were, however, found within the lungs of many of the diabetics, confirming earlier studies by Baxter and Ashworth. One hypothesis we have put forward suggests that intravascular plugs of fat may not be embolic but instead may have formed *in situ*, by coalescence of the smaller fat droplets in the hyperlipemic diabetic plasma; an event particularly likely to occur in these capillaries from which considerable fluid is normally removed from the circulating blood (as in glomeruli and retinae), thereby increasing the concentration of retained fat within the vessel. Considerable loss of fluid from capillaries of the retina is known to occur physiologically and may be intensified

in times of circulatory embarrassment. Resultant concentration of hyperlipemic plasma might conceivably play a role here in the pathogenesis of diabetic retinopathy analogous to that proposed above in the case of focal glomerular sclerosis.

A relationship has been demonstrated between the incidence of retinal lesions in diabetics and elevation in their plasma levels of macromolecular lipoproteins (S_f 12–20) class by ultracentrifugation.

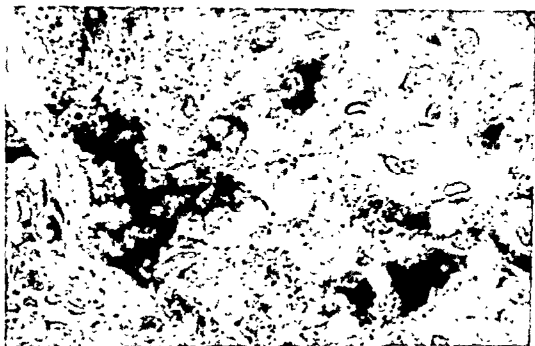


Fig. 26-5. Small and large plugs of fat *(black) fill many of the capillary loops of this glomerulus present in a frozen section stained with Oil Red O prepared from the kidney of an adult diabetic with Kimmelstiel-Wilson disease. Much of this lipid is clearly intralumenal.*

Two independent investigators (Morgan in England and Urbanik in Germany) have reported several points of resemblance between the fundal changes in traumatic fat embolism and that in diabetes. Cook in England later showed that biweekly injections of fat intravascularly into cats for periods of one week to two months produced capillary dilatations associated with intracapillary obstruction by globules or cylinders of fat. In some of his animals the lesions persisted up to six months following the last injection. The changes did not duplicate precisely, however, those of diabetic retinopathy, suggesting that other factors and mechanisms must play a role in man. Studies shortly to be published from this laboratory (Pope and Hartroft) on a small series of

eyes removed from diabetics at autopsy have revealed plugs of fat within the aneurysmal dilatations of retinal capillaries as well as lipid within the wall. The appearance in whole mounts of these retinae indicate that bulging of the endothelium occurs as a herniation between the supporting reticular and basement membrane fibers, suggesting a sequence secondary to obstruction caused by lipid plugs "downstream" from the aneurysm.

SUMMARY AND CONCLUSIONS

The available data clearly demonstrate the existence of profound disturbances of fat metabolism and transport in diabetics, particularly those of the older age groups. These abnormalities are reflected morphologically by the presence of considerable amounts of fat in pathologic islets, glomeruli, and retinae. Although a number of hypotheses have been outlined in this chapter to suggest that the masses of abnormal lipid may have pathogenic significance, this interpretation is by no means the consensus of pathologists who have studied these lesions. The writer cannot overemphasize that neither the reader nor he should regard these notions as other than mere working hypotheses, useful only insofar as they may stimulate attempts to obtain additional data that might serve to disprove or establish the importance of the lipid. One of the greatest handicaps in pursuing this line of investigation is our present inability to produce at will close morphologic counterparts in animals of all these lesions of diabetics seen frequently at the autopsy table. If these lesions could be reproduced in animals, the gateway would be opened to illuminating studies of their pathogenesis, utilizing all the resources currently available to the experimental pathologist, including electronmicroscopy, histochemistry, and microenzymology.

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Chapter 27

THE INCIDENCE AND DEVELOPMENT OF DIABETES MELLITUS

Harvey C. Knowles, Jr.

INTRODUCTION

In recent years much attention has been directed toward the epidemiology of diabetes. Attempts have been made to determine the incidence of diabetes, disclose factors contributing to its development, and characterize a prediabetic state. Interpretations have been made of data collected from large groups of diabetic and control subjects. Unfortunately, in this type of study it cannot always be certain that data are entirely accurate or that comparable populations are being tested. Despite these limitations, certain patterns have become apparent, and hypotheses have been advanced accordingly.

THE INCIDENCE OF DIABETES

Incidence in the United States

Studies of the occurrence of diabetes in the United States have been conducted by various means. An incidence of 0.5 per cent of mellituria was noted in examinees for life insurance. Door-to-door surveys raised

this occurrence rate to 1.0 per cent. In voluntary detection programs involving actual testing, incidences ranging from 0.83 per cent to 4.0 per cent were reported. However, though these types of programs serve to disclose the unknown diabetic, they are not wholly satisfactory in determining the true incidence of diabetes. Those tested do not necessarily represent a true cross section of the population, and known diabetics may not participate. In a study designed to avoid these objections, Wilkerson and Krall investigated the incidence of diabetes in Oxford, Massachusetts, in 1946–47. Efforts were made to include the entire population of this town. Postprandial blood glucose concentrations were determined, and tolerance tests conducted when indicated. The age distribution of those tested compared favorably with that of the town as a whole and with that given for the national population. There were 40 known and 30 unknown diabetics found in 3,516 persons tested from a town population of 4,983, an incidence of 1.99 per cent in the participants.

It appears reasonable to assume that currently in this country at least 1.0 per cent of the population has known diabetes, and another 1.0 per cent has diabetes undiagnosed as yet. This estimate is probably conservative and eventually may need revision. In addition, there is an approximate 3.0 per cent incidence of potential diabetics. The incidence is lower in younger age groups. Investigations of draftees have yielded an occurrence rate of 1.0 per cent, and of college students, 0.47 per cent. Diabetic school children are estimated to consist of about 5.0 per cent of the total diabetic population, or about 0.1 per cent of the population at large. Reports from clinics dealing with childhood or "juvenile" diabetes place the occurrence of diabetes in infancy (less than one year of age) as 0.4 per cent to 3.0 per cent of the diabetic clinic attendance.

Incidence in Other Populations

The occurrence of diabetes in other parts of the world has not been investigated to the extent of that in the United States. A study in two small Canadian communities disclosed an incidence of 1.3 per cent of known and unknown diabetics. However, it was not certain that all known diabetics in the communities participated. Estimates of approximately 0.5 per cent incidence were determined from the food registries of Stockholm and West Berlin in the postwar years.

There is insufficient information to demonstrate convincingly that racial differences exist in susceptibility to diabetes. In the studies in this country, it would appear that the Negro and white populations are afflicted in comparable degree. It has generally been held that the

Wilder, and Thompson and Watson lend support to this hypothesis. If the recessive gene leading to the susceptibility of diabetes is designated as "d" and its normal allele "D," the population would include "dd," the diabetic or potential diabetic, "Dd," the carrier, and "DD," the person with no diabetic trait. According to this scheme, the mating of two diabetic or potential diabetic subjects would yield progeny who would all be diabetic or potentially so. The remaining possible combinations produce issue as follows:

Carrier × Person without diabetic tendency	*Carrier × Carrier*	*Carrier × Diabetic*
(Dd) ↓ (DD)	(Dd) ↓ (Dd)	(Dd) ↓ (dd)
2 Carriers 2 Persons without diabetic tendency	1 Diabetic 2 Carriers 1 Person without diabetic tendency	2 Diabetics 2 Carriers

Utilizing the hypothesis of a recessive autosomal gene and assuming the known diabetic population to be 1.0 per cent, Steinberg and Wilder have estimated that the diabetic gene is present in from 20 per cent to 25 per cent of the total population, and that the total diabetic population, diagnosed, undiagnosed, and potential approaches 5.0 per cent. However, it is also possible that in certain pedigrees other means of transmission may be present. In a remarkable genealogy reported by Burnstein and Patterson there was suggestion of a dominant gene.

Woodyatt and Spetz have advanced the hypothesis of anticipation. In this phenomenon, diabetes would be inherited with the mean age of onset continuously earlier in successive generations. In their observations, the mean difference of onset ages between generations was 20 years. Accordingly, a patient with onset at age 40 might produce a child with onset at age 20. In the next generation the onset would be at birth, or theoretically before birth, with termination of the strain. Steinberg and Wilder have critically examined this hypothesis and have concluded that though there may be a tendency for prior development, the association is highly irregular, and the observation is only of statistical rather than of biologic value.

The Factor of Environmental Stress

Numerous factors have been listed to precipitate diabetes in persons with the inherited tendency. It has been suggested that the mere burden of living may be sufficient. However, attention has been directed in the main toward stresses involving some phase of carbohydrate metabolism. These stresses include general conditions such as diet and obesity, pregnancy, infection, pancreatic disorders, and hyper-

glycemic states associated with endocrine disturbances other than in the pancreas, and disorders of the nervous system.

DIET AND OBESITY. Attempts have been made to relate the development of diabetes to dietary patterns. There is no conclusive information relating diabetes onset to carbohydrate consumption. However, there is some evidence to suggest indirectly a relation between the incidence of diabetes and the consumption of diets high in calories and fats. In countries deprived of food in World War I as well as in Japan and Western Germany during World War II, there were apparent decreases in the incidence of diabetes. The lowered rates of occurrence correlated with decreases in dietary calories and fat. However, it cannot be stated whether the decrease in fat intake, a decreased incidence of obesity resulting from the low calorie diets, or unrelated factors may have been responsible for the apparent diminished incidence of diabetes.

The relation between obesity of dietary origin and the development of diabetes has long been recognized. Approximately 40 per cent of the diabetics in this country and in England have been described as being 20 per cent or more overweight at the time of diagnosis of diabetes. It is estimated that 10 per cent to 30 per cent of the population of the United States is 10 per cent overweight. However, despite the common finding of obesity in the new diabetic, the proportion of overweight subjects who develop diabetes is probably not large. Because of the relation of obesity to diabetes, it has been suggested that obesity may be genetically transmitted, and accordingly some diabetic families will be obese and others thin. However, proof of this is lacking, and common environmental factors could explain familial obesity equally as well as an inherited trait.

PREGNANCY. That pregnancy can affect carbohydrate metabolism in the normal person is clear from observations of an impaired glucose tolerance developing during pregnancy and returning to normal after delivery. Arguments favoring a relation of diabetes to prior pregnancy have been based on studies purporting to show an increased rate of parity in the prediabetic female, a decreased incidence of diabetes in the single as compared to the married female, and an increase in the incidence of diabetes in females in excess of males after the age of 40. These arguments must remain tentative at present since control groups in the studies were not always of a strictly comparable nature, and it has not yet been conclusively demonstrated that diabetes is more common in females. Furthermore, the findings of increased parity could also be explained by increased fertility in the prediabetic female. Evidence has recently been presented that the menarche may occur early in sub-

jects who develop diabetes after age 18, and it was suggested that increased parity might be related to this development.

INFECTION. Diabetes is frequently diagnosed when a patient is hospitalized for an infection. Patients may be seen with infection and ketoacidosis who had no prior symptoms of diabetes. Consequently, it is frequently stated that diabetes may be initiated by an infection. It is true that in some instances a patient may have a normal glucose tolerance and a month later be hospitalized with ketoacidosis and infection. However, such instances have also occurred without evidence of infection. There is no evidence that infections per se can cause diabetes. It is more likely that infection may aggravate latent diabetes so that the patient seeks medical attention for diabetic symptoms, or that asymptomatic diabetes is discovered when a patient seeks attention for his infection.

PANCREATIC DISORDERS. Insulin insufficiency and hyperglycemia occur in the course of pancreatic disorders such as chronic pancreatitis and hemochromatosis. Such patients are regarded as diabetics and managed accordingly. As mentioned previously, it is not known if these conditions lead to the same incidence of degenerative complications seen in the inherited form of diabetes mellitus. Nevertheless, it is thought by some that the induced hyperglycemia may establish the complete syndrome of diabetes in the predisposed subject. In a clinicopathologic study of hemochromatosis, no vascular lesions peculiar to diabetes were found. Isolated reports of retinopathy in diabetes due to chronic pancreatitis and pancreatectomy have appeared, but it cannot be determined that these patients did not inherit the diabetic tendency. Of unusual interest is a recent report from the Mayo Clinic describing retinopathy 3 years after pancreatectomy for an islet cell adenoma in a 36-year-old woman without family history of diabetes. Similar conjectures may be made concerning the diabetes associated with overactivity of the thyroid, adrenal, and pituitary glands, or during adrenal steroid therapy. Retinopathy has been reported in 2 of 21 patients with Cushing's syndrome and sustained hyperglycemia, and in 3 of 21 patients with acromegaly and hyperglycemia. At present, the observations are too limited in number and time to determine if prolonged hyperglycemia induced by these states can lead to diabetic vascular complications with occurrence consonant with that of the inherited tendency to diabetes.

DISORDERS OF NERVOUS SYSTEM. It has been postulated that disorders of the nervous system may be concerned with the development of diabetes. Transient hyperglycemia is seen occasionally in the course of cerebral injury or vascular accident However, no unusual incidence of

diabetes has been noted in patients with brain tumors, hypothalamic disease, or other types of cerebral pathology. Similarly, disturbances of the psyche have been invoked as predisposing agents. It has been suggested that the personality of the diabetic is different from that of the nondiabetic, that diabetes might be initiated by a specific emotional upheaval, and that stressful life situations over a long period might cause sufficient hyperglycemia to bring diabetes to light in the susceptible person. However, other investigations in groups of patients have not been in agreement with the first two concepts. In addition, the studies of Hinkle and Wolf would indicate a lowering of the blood glucose concentration rather than an elevation during acute emotional stress unless sufficient fear or anger was present to elicit adrenalin secretion. Further study is needed to affirm or deny a role of the nervous system in the development of diabetes.

In many instances of diabetes no factors of environmental stress are apparent prior to the onset. It is very likely that many stresses are as yet unknown. Some factors may be entirely unrelated to carbohydrate metabolism. It is also conceivable that heredity factors other than the diabetic tendency are concerned with the development of overt diabetes.

THE PREDIABETIC STATE

In recent years much has been written about the prediabetic state. The recognition of this state with proper education of the patient would be of tremendous importance in reducing the incidence of some of the preventable complications of diabetes such as ketoacidosis or advanced foot ulcers.

The prediabetic state is usually considered to be the period prior to the development of clinical diabetes when glucose tolerance is normal or slightly impaired, but certain phenomena common to diabetes may be seen. A family history of diabetes automatically renders the patient a suspect, and the presence of obesity would increase the chance of development. It is not uncommon for the prediabetic to present minor derangements in carbohydrate metabolism before clinical diabetes develops. A minimally impaired glucose tolerance test may be present for many years before the curve becomes that of the diabetic. In prolonged observation of patients with mild hyperglycemia, incidences of development of overt diabetes of over 50 per cent have been found. The prediabetic will occasionally show a diabetic glucose tolerance curve during stress which returns to normal after subsidence of the stress. Examples may be found in the course of fever, pregnancy, or injury.

Fajans and Conn have made use of this observation to demonstrate a diabetic glucose tolerance test after cortisone administration in otherwise normal relatives of diabetics (see Chap. 30). These same authors have recently reported that renal glycosuria may be a precursor of the diabetic state, and that spontaneous hypoglycemia may occur early in the course of diabetes. However, it is not certain if the hypoglycemia may be present before glucose intolerance sets in.

Of particular interest has been the observation that abnormalities of pregnancy in the diabetic may be seen also in the prediabetic (see Chap. 30). The average weight of the diabetic infant exceeds that expected for the period of gestation. There is a high rate of stillbirths in diabetic pregnancies, and this increased rate has been shown to occur in the prediabetic as well. There is some evidence that the birth weights of infants of prediabetic fathers may be increased. Finally, there have been noted isolated instances of some of the complications of diabetes such as diabetic glomerulosclerosis and neuritis occurring before the development of a diabetic glucose tolerance test. It is likely that other characteristics of the prediabetic will be described in the future.

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Chapter 28

EARLY CLINICAL PICTURE OF DIABETES

Garfield G. Duncan

INTRODUCTION

The beginning of the diabetic state is obscure. However, partly from conjecture and partly from fact, primary or essential clinical diabetes as distinct from secondary diabetes can be considered to emerge as the product of a complex and abnormal situation initiated by a hereditary predisposition and influenced unfavorably by certain circumstances, normal and abnormal. For practical purposes and for the sake of simplicity, the evolutionary processes and the period between the obscure inception of diabetes and the time that this disorder is identified clinically may be divided into several phases:

1. The dormant period, as illustrated in the individual predisposed to diabetes by heredity and during which there are no detectable evidences of diabetes. But during this period normal influences, notably growth, hormonal maturation, increases in body weight, and factors that increase the production of steroids exert deleterious effects in carrying obscure processes toward that phase when disturbances in the metabolism of carbohydrate are detectable.

2. A phase in which a relative insensitivity to insulin prevails. In studies of sensitivity to insulin in our clinic at the Pennsylvania Hos-

pital all diabetic patients were found to be relatively insensitive to insulin (5) (Fig. 28-1). However, only 38 per cent of women who had a family history of diabetes and those who had given birth to infants weighing more than 9 pounds revealed what was considered to be a relative insensitivity to insulin, as did only 12.5 per cent of men and women presumably normal and with no family history of diabetes.

Those individuals who have diabetic type of responses to glucose tolerance tests after the administration of steroids (6, 9) (Fig. 28-2),

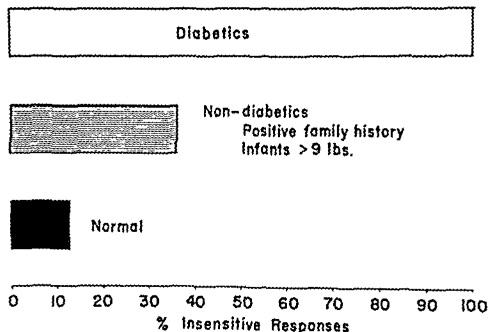


FIG. 28-1. Insensitivity to insulin. Glucagon-free insulin sensitivity tests indicate that all adult diabetics' tests showed relative insensitivity to insulin. Thirty-eight per cent of nondiabetic women who had a family history of diabetes and who had given birth to infants weighing more than 9 pounds were relatively insensitive to insulin in contrast to 12.5 per cent of presumably normal men and women (5).

or during pregnancy (7, 10) or other complication that increases the production of steroids, are considered to be in this phase of the prediabetic state. In this phase an abnormally high incidence of recurrent abortions and stillbirths occurs. A less well established indication of the prediabetic phase is the occurrence of hypoglycemic episodes (2, 8), increased hunger, and excessive gain in weight. In one instance observed by this author, the hypoglycemic episodes were so severe as to be indistinguishable from those encountered in cases of functioning islet cell tumors of the pancreas and yet within a few months a transition to a severe labile diabetes occurred.

3. That period when there is a diabetic type of response to a glucose tolerance test under usual conditions, that is, without the impact of steroids, pregnancy or infections.

4. The phase in which there are clinical manifestations of diabetes with the development of more or less characteristic symptoms and,

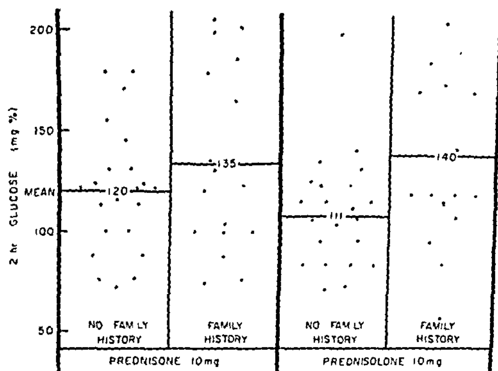


Fig. 28-2. Results of steroid-glucose tolerance tests (Somogyi-Nelson). A family history of diabetes reflects unfavorably in this test when either prednisone or prednisolone was given prior to the ingestion of the glucose. (Reprinted by permission of K. M. West and the American Diabetes Association (9).)

sooner or later, complications. The diagnosis of diabetes is most frequently made during this last phase. Many diabetic patients have no symptoms of diabetes in its early clinical course and are unaware that they have diabetes until it is detected during examinations for insurance, periodic clinical surveys, or during the course of an acute complication. Such subjects present relatively barren prospects for a study of the pretreatment phases of diabetes. On the other hand, patients who have subjective evidences of this disease provide the basis for the evaluation of the manifestations of diabetes during the period between the onset of symptoms and establishment of the diagnosis.

ONSET AND PROGRESSION OF CLINICAL DIABETES

The onset of the symptoms of diabetes may be acute. One adult patient first detected polyuria and thirst while enroute by plane from one island in the Pacific to another. The abrupt onset in which the symptoms are detectable within a few days to a few weeks is unusual, occurring in less than 2 per cent of cases. It is encountered almost exclusively in the juvenile patient. The onset of bed-wetting in the very young patient may indicate the approximate date of the onset of diabetes. The abrupt appearance of this symptom, associated with decreasing body weight, indicates the acuteness of the onset in many juvenile patients. Unrecognized and untreated, the disease in these patients pursues a rapid course, as testified to by a life expectancy of approximately two years in the preinsulin eras.

In contrast, the onset in adults is usually indefinite. This is especially true in those with mild and relatively mild diabetes, who constitute approximately 80 per cent of the diabetic population. Between these extremes of abrupt and indefinite onset are those patients who can approximate the date of onset of symptoms with varying degrees of precision.

Acute complications and notably acute infections are prone to precipitate an abrupt or sudden onset of the manifestations of clinical diabetes in all grades of severity of the disorder. The symptoms of diabetes may be more or less obscured by those of the acute disorder; they may disappear with correction of the latter, and unless the patient is receiving professional medical care the diabetes may escape detection during such episodes.

The average interval between the onset of symptoms and diagnosis in 405 of Danowski's child diabetics was 82 days with variations from 3 days to 24 months. In 277 (68.4 per cent) of these children the diagnosis was made within 30 days of the onset of symptoms. This is in contrast with approximately 10 per cent of adults whose diabetes is of rapid onset.

Characteristically, children manifest symptoms of diabetes at the time the diagnosis is made, whereas only 34 per cent of adult diabetics admit, and 23 per cent deny having symptoms of diabetes at the time the diagnosis is made (3).

There is no way of knowing when diabetes begins. One might ask whether being a mother of an 11-pound baby is a manifestation of diabetes in the case of a woman who in later years develops hyperglycemia and glycosuria. The generally accepted concept of the nature of diabetes is that there must be evidence of disturbances in the metabolism

of carbohydrate. One might predict that this concept will change when basic prodromal changes are understood to include manifestations such as occur in the case of the mother of big babies, or the mother with a family background of diabetes who has recurrent abortions or stillbirths, the occasional typical diabetic retinitis, or the less well defined neuropathies that have been observed prior to the appearance of the disturbance in the metabolism of carbohydrate.

The progression of the diabetic state from the initial disturbances in the metabolism of carbohydrate to the onset of symptoms is poorly understood, but the rate and degree of this evolution is influenced by growth, age, body weight, pregnancies, corticoid therapy, and disorders *accounting for excessive production of corticoids, and infections. In its* early phases, and notably in adults, diabetes may not be suspected and may escape detection until the patient seeks treatment because of chronic complications of the diabetes, notably degenerative disorders —diabetic retinitis, peripheral vascular disease, coronary insufficiency, and renal disease. Diabetes is frequently detected while the patient is under treatment for an acute complication that may or may not bear some etiologic relationship to the diabetes.

CLASSIFICATION OF DIABETES

The objective and subjective manifestations of diabetes are manifest in a variety of forms, even in the pretreatment phase. Based on characteristics common to different types of diabetes, the classification presented in Table 28-1 has proved of practicable value.

Primary or Essential Diabetes

This form of diabetes is encountered in two types, that acquired in adult life and that which appears in childhood or in youth and often is designated as the juvenile type. These two types differ greatly in the pretreatment phase (Table 28-2).

ADULT TYPE DIABETES: GENERAL CONSIDERATIONS AND SYMPTOMATOLOGY. The adult type, as the name indicates, appears in adults and characteristically is a relatively stable diabetes which often exists for long periods—years—without symptoms. The adult type of diabetes may be subdivided in keeping with (a) the sensitivity to sulfonylurea compounds in more than two thirds, and (b) the lack of this sensitivity in less than one third of these cases.

Most subjects acquiring clinical diabetes in adult life, approximately 82 per cent of the diabetic population, are or have been overweight when they seek treatment. These patients, though their diabetes is usu-

ally mild, are relatively resistant to insulin. Insulin in excess of 100 units may be necessary to control the diabetes unless the total calories consumed are below the total necessary to keep the body weight constant. It is significant that despite the tolerance of such large amounts of insulin, this is not an indication of the severity of the diabetes, which is readily controlled by a reduction in body weight by decreasing the

TABLE 28-1. A CLINICAL CLASSIFICATION OF DIABETES MELLITUS

I. [illegible]
    [illegible]
        Overweight is usual (in excess of 80 per cent of these patients)
        Relatively insensitive to insulin
        Not prone to develop ketosis, in the absence of acute complications
    B. *Unstable*, juvenile type
        Onset usually in childhood or youth
        Very sensitive to insulin
        Very susceptible to ketosis
II. Secondary diabetes
    A. [illegible]
        2. Medullary—pheochromocytoma
    B. Hyperpituitarism
        1. Acromegaly
        2 Pituitary basophilism
        3. Therapy—adrenocorticotrophic (ACTH) and growth hormones
    C. Hyperthyroidism (rarely, if ever, a cause of clinical diabetes)
        1 Thyrotoxicosis
        2 Thyroid therapy
    D Destruction or excision of islet tissue
        1. Hemochromatosis
        2 Pancreatitis
        3 Cystic disease or neoplasm of pancreas
        4 Removal of pancreas
        5 Trauma to pancreas (?)

caloric intake, a concept clearly established by Allen (1) and illustrated in Figure 28-3.

The obese adult diabetic patient who has received no treatment for his metabolic disorder has a mild diabetes. During acute complications, however, the mildness may be temporarily obscured by the demands incident to the complication. Proof of the mildness of the diabetes is demonstrable when the glycosuria and hyperglycemia are observed to subside as the body weight is reduced by restricting the caloric intake. No such benefit accrues from the loss of weight frequently observed in the pretreatment phase, attributable to loss of nourishment in the form

TABLE 28-2. CONTRASTING CHARACTERISTICS OF THE TWO TYPES OF PRIMARY OR ESSENTIAL DIABETES

	Juvenile	*Adult acquired*
Incidence	Relatively infrequent	Common
Onset	Acute	Gradual
Interim between onset of symptoms and diagnosis	Short; weeks	Long; months to years
Age at onset, in years	Prior to 15 (usually)	After 10 (usually)
Body weight at onset	Normal or below	Overweight in approx. 82 per cent of cases
Degree of loss of weight in pretreatment phase	Marked	Slight to marked
Fasting sugar in pretreatment phase	Elevated	Frequently normal
Response to glucose tolerance test	Slight if any decrease in blood sugar between second and third hour	Decrease in blood sugar between second and third hour is usual
Stability of diabetes	Unstable	Stable
Sensitivity to insulin	Very sensitive	Relatively insensitive
Sensitivity to sulfonylureas	Insensitive	Sensitive
Sensitivity to develop ketosis	Very sensitive	Relatively insensitive
Effect of exercise	Intensifies lability	Little effect on lability
Remission of diabetes (transitory)	Not rare	Rare, if ever
Evidences of degenerative changes in pretreatment phase	Uncommon	Common
Rapidity of development of degenerative changes	Rapid	Slow
Chronic infections in urinary tract	Rare	Common

of glycosuria. As the weight decreases under these circumstances, it does so without the favorable influence that restricted caloric intake has to offer; hyperglycemia and glycosuria do not subside, when thinness results the diabetes has become severe and insulin or other drug therapy will usually be imperative, and the advantage that might have been gained by a judicious reduction in weight by restricting the diet has been permanently lost. Tolbutamide suffices to control the diabetes in some of these patients, but its effectiveness is much less apparent and less likely to be permanently effective than if it is given before the patient becomes underweight.

The overweight adult diabetic patient in the pretreatment phase is not, barring acute complications, prone to develop ketosis. This relative immunity to ketosis disappears, however, in the presence of acute complications, notably acute infections.

CLINICAL FEATURES. In a study of 500 consecutive diabetic patients, Beaser has indicated the relative frequency of the symptoms and other

clinical features encountered (Table 28-3). The presenting complaints may be subdivided according to whether they are considered to be typical of (1) uncomplicated diabetes: polydipsia, polyuria, loss of weight, weakness, pruritus, nocturia, polyphagia, visual changes, headache, anorexia, drowsiness, or (2) complications commonly seen in

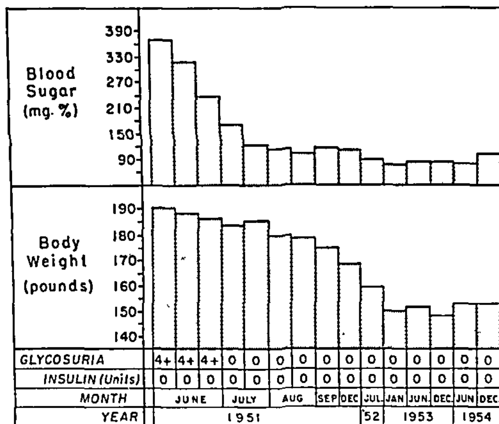


FIG. 28-3. Typical effect of therapeutic undernutrition in obese diabetics. The clearing of glycosuria and the correction of the hyperglycemia are associated with the reduced diet and the decrease in body weight. (The data are from the record of a patient treated in the Out-Patient Clinic at the Pennsylvania Hospital (5).)

diabetics, notably those indicative of changes in the vascular system and involving chiefly the eyes, the coronary circulation, the kidneys, and the peripheral circulation; infections notably of the skin and urinary tract, and involvement of the nervous system, accounting for the bizarre manifestations of diabetic neuropathy. Symptoms of these disorders will be considered in greater detail in other chapters. They are mentioned here because singly or in combination the disorders may account for symptoms for varying periods in the pretreatment phase.

Degenerative disorders considered to be characteristic of diabetes are found for the most part in primary or essential diabetes.

Diabetes that is secondary to other disorders (Table 28-1) is not nearly as likely to cause degenerative changes and when it does, these changes are not nearly as advanced as one commonly finds them in primary diabetes at the time the diagnosis is made. It may be that this relative infrequency of degenerative disorders in secondary diabetes is in direct relationship to the relatively short duration of the diabetes, as is usually the case. Patients with secondary diabetes characteristically have no family history of diabetes. Also, it is likely that the prodromal phase during which diabetes could not be detected is short, a direct relationship to the duration of the disorder causing the diabetes.

TABLE 28-3. CLINICAL FEATURES AT THE TIME OF DIAGNOSIS*

Presenting complaint	*Total number*	*Percentage of group*	*Percentage of total*
Diabetic group			
Polydipsia	32	49 0	16 8
Polyuria	30	46 0	15.8
Weight loss	30	46 0	15 8
Weakness	25	38 0	13 1
Pruritus	23	32 0	12 1
Nocturia	9	14 0	7 4
Polyphagia	7	11.0	3 7
Refractive changes	4	6.0	2 1
Headache	2	3 0	1 0
Anorexia	1	1 5	0 5
Drowsiness	1	1 5	0 5

* Partial compilation extracted from Beaser (9)

The physical abnormalities observed in the adult patient and related to the diabetes per se are for the most part restricted to evidences of loss in weight, whereas the multitude of evidences of degenerative changes and other complications are common. Some evidences of vascular disease—retinitis, atherosclerosis, coronary insufficiency, renal disease, and peripheral vasicular disorders—singly or in varying combinations are detectable in most adult diabetic patients at the time that the diagnosis of diabetes is made.

JUVENILE TYPE DIABETES: SYMPTOMATOLOGY. Symptoms complained of in cases of the juvenile type of diabetes, as observed in children, young adults, and a relatively small percentage of older patients are, in order of frequency, polyuria, polydipsia, loss in body weight, fatigue, polyphagia, nocturia, and irritability. Infections may complicate the

clinical picture and when the patient is ketotic symptoms characteristic of this complication are noted. Especially common are increasing polyuria, fatigue, anorexia, nausea and vomiting, abdominal pain, and air hunger.

At the time that the diagnosis is made the young patient is usually relatively free from the stigmata of degenerative disorders. Exceptions to this rule are rare. In the uncomplicated state, evidences of loss of weight and mild degrees of dehydration are the characteristic findings. Abnormalities associated with complications, that is, infections and ketosis, are considered elsewhere. Features in which the juvenile type of diabetes differs from the diabetes appearing in adults are presented in Table 28-3.

SUMMARY

The patient with the juvenile type of primary diabetes, in general, gives a history of having an acute onset of symptoms that have been of relatively short duration. The patient is usually young, is not overweight, develops ketosis easily and rapidly. The fasting blood sugar is elevated in the pretreatment phase and evidences of degenerative disorders are infrequent. This type of diabetes is labile. All these patients require insulin, to which they have a marked sensitivity, but tolbutamide therapy is ineffective. The lability of the diabetes is intensified by physical exercise. Transient remissions of the diabetes, though uncommon, are not rare. Though evidences of degenerative disorders are absent or minimal when the diabetes is detected, these patients are prone to develop degenerative vascular complications, which progress at a more rapid rate than when associated with adult-acquired diabetes.

The adult-acquired or stable type of diabetes is approximately eighteen times as frequent as the juvenile type. The diabetes is detected after 40 years of age in the great majority of these patients. The onset is gradual and there is usually a prolonged symptom-free period. Approximately 82 per cent are, or have been, overweight and many have lost weight. Patients with adult-acquired diabetes for the most part are not prone to develop ketosis, barring acute complications; the diabetes is relatively stable, it is less sensitive to insulin, and exercise does not exert such rapid or profound effects as in the juvenile type of diabetes. Complete remission of the diabetes is exceedingly rare though an appropriate reduction in weight does in some instances force the diabetes so far into the background that it is not detectable until an acute complication, steroid therapy, or a gain in weight brings it to the fore. Normal fasting blood sugar values are common in the obese diabetic patient.

Degenerative complications are common at the time the diabetes is detected, but the progression of these abnormalities is slow in contrast to those seen in the juvenile type. Infections in the urinary tract are common, occurring in excess of one third of the female patients. In at least two thirds *of the cases of adult-acquired diabetes the glycosuria* and hyperglycemia are *controllable by tolbutamide therapy.*

In secondary diabetes owing to excessive hormonal influences or to ablation or destruction of the pancreas, the outstanding characteristics are usually those of the primary disease (Table 28-1) (see Chap. 47). Also, if it is practicable to remove the cause in secondary diabetes, e.g., corticoid therapy or tumors producing excessive corticoids, a cure of the diabetes may be anticipated with reasonable certainty. Characteristically, the degenerative disorders frequently associated with primary or essential diabetes are not present when the diagnosis of secondary diabetes is made. The symptomatology associated with the diabetes per se is the same in primary and secondary diabetes.

The pretreatment phase logically terminates when the diagnosis of diabetes is established by methods outlined in Chapter 30.

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Chapter 29

JUVENILE DIABETES

Priscilla White

Incidence

Juveniles, defined as those recognized under age 15, comprise 5 per cent of the world's diabetics. Their incidence in the childhood population is 1 per 2,500 individuals. Their sex distribution, unlike that of older age groups, is nearly even. Among 4,054 Joslin Clinic juvenile patients, 51.4 per cent were females and 48.6 per cent males. The peak for age at onset in girls, at 10 years, coincides with their earlier maturity. The peak for age at onset in boys is 13 years and for all our children 11 years.

Etiology

That the tendency to develop diabetes is inherited is shown best in childhood diabetics. Because of the many opportunities to recheck family histories, 57 per cent of our juveniles revealed positive family histories after 20 years of diabetes. Transmission through recessive genes is suggested by significant differences in the frequency of diabetes in the offspring of one, compared with two diabetic parents, 22 per cent for the former, 62 per cent for the latter.

Trauma rarely (0.1 per cent), obesity seldom (5 per cent), infections

sometimes (10 per cent), and spurts of linear growth commonly (90 per cent) precede diabetes onset.

Natural Course

Juvenile diabetes follows a significant natural course. After an acute onset with frequent recognition in coma, independently of any special antidiabetic therapy, a favorable remission phase occurs. This is recognized clinically in one third of all cases and has been documented recently by the demonstration of greater than normal blood insulin-like activity. This remission phase, appearing some three months after the initiation of treatment, normally lasts three to twelve months. Intensification of diabetes follows linear growth, infections, and puberty. Increase in intensity of the degree of diabetes to a total diabetic state in childhood is supported by the following evidence: the gradual increase in requirement for insulin from 0.25 to 0.5 unit per pound of body weight; the absence of insulin in pancreatic extracts (14); the absence of insulin-like activity in the blood (2); the disproportionately small size of the pancreas for total body weight (3); diminution of numbers and weight of islets (10); and absence of insulin granules (1); and, finally, the response to sulfonylureas, which is in inverse ratio to the duration of diabetes, 90 per cent responding in the first six months of diabetes, 6 per cent only after five years (4). All of this implies that the total or near-total diabetic state is eventually acquired in the juvenile patient.

In contrast to experience with adults, the continuous perfect correction of the metabolic defect without chemical overcorrection is almost impossible. The search for ways to alter the natural course of diabetes or to potentiate the action of insulin is, therefore, imperative.

Despite the profound degree of this metabolic defect in childhood, linear growth proceeds at satisfactory rates; the menarche is delayed but eventually fertility is unimpaired.

During the course of their diabetic lives, juveniles are exposed to three potentially lethal complications. Eventually, after 20 years, coma has occurred in 50 per cent, sepsis in 30 per cent, and vascular damage in 94 per cent (13). Insulin, antibiotics, and chemotherapy have solved the lethal aspects of coma and sepsis, in 97 per cent of the patients having the former and in nearly 100 per cent of those complicated by the latter. This leaves vascular disease the main problem and its prevention the primary objective of treatment.

Vascular Damage

The clinical, pathologic, and physiologic features of vascular disease in juvenile diabetes are many. All types of vessels are involved in all

kinds of sclerosing processes, but the involvement of the ubiquitous arteriole, venule, and capillary constitutes the disabling and lethal problem of the juvenile.

CLINICAL FEATURES. A well-defined order for appearance of the lesions characterizes the juvenile. Retinopathy precedes calcification of arteries, and they in turn usually precede proteinuria, whereas hypertension develops late. The lesions are rarely recognized before age 20 or a duration of 10 years, but by 15 years' duration 20 per cent, and by age 30, 60 per cent show at least minimal vascular changes.

The incidence of the sequelae of vascular damage in a series of 1,072 20-year survivors was as follows: uremia in 9 per cent, blindness in 6 per cent, myocardial infarction in 6 per cent, cerebrovascular damage in 2 per cent, and gangrene in 0.5 per cent.

PATHOLOGY. The histologic changes of the small blood vessels include hyaline thickening of the intima of the arteriole, phlebosclerosis, beading and tortuosity of venules and hyaline thickening, endothelial proliferation, and microaneurysms of the capillaries (12).

PHYSIOLOGY. In addition to the evaluation of these structural changes, much may be learned from the behavior of the small vessels in vivo; the affectors and correctors of abnormal degrees of dilatation and constriction; the results, intravascular and perivascular, of abnormal responses; the degree of reversibility; and irreversibility of the responses. Such studies have been made in our patients by Jorn Ditzel (5). The subjects included nondiabetic controls, untreated and treated diabetics, long-term and short-term cases, and offspring of diabetic mothers.

Two distinctive patterns were observed. Pattern I, found in untreated, short-term cases and offspring of diabetic mothers, showed venular dilatation, arteriolar narrowing, capillary irregularities, intravascular sludging, perivascular edema, and hyalin deposition. Pattern II consisted of arteriolar constriction, venular narrowing, capillary obliteration, intervascular sludging, and perivascular edema. This pattern occurred in acidosis and in long-term diabetes. These responses remain reversible for long periods of time in the bulbar conjunctiva. Comparable irreversible lesions are inferred in the retina, kidney, nerva vasorum, vaso vasorum, tissue hypoxia, and starvation resulting.

Venular dilatation may be produced by lack of insulin or of cortisone. Arteriolar constriction may be produced by cortisone, oxygen of high tension, decrease in pH and CO_2. Arteriolar tone may be restored by CO_2 of high tension, O_2 of low tension, or high values of pH. Venular tone may be restored by insulin and biguanides.

CAUSES. The positive correlation between lack of control of diabetes and precipitation of and acceleration of the vascular lesions in the Joslin Clinic has been reported by Keiding, Root, and Marble and has been

reported by Jackson and Hardin, Dunlop, and others. The possible theoretical relationship includes arteriolar constriction resulting from the low CO_2 and pH in acute or chronic acidosis, as well as the cortisone effect at that time. Hyperglycemia per se may be implicated as the means of delivery of O_2 at high concentration at cellular level. Chronic hypoinsulinism is implicated in venular dilatation.

Treatment

The chemical control of diabetes in childhood has for its objective normoglycemia in the fasting state, glycosuria amounting to less than 5 per cent of carbohydrate intake measured in grams of glucose excreted in 24 hours, normal blood lipids, and freedom from ketosis.

To achieve control, a wide variety of insulins may be used. The initial prescription depends upon size; if ketosis is absent, 0.25 unit per pound of body weight may be used. The choice of the Joslin Clinic is NPH. In only 10 per cent is control achieved by this alone; 40 per cent require a mixture of regular with NPH insulin; 50 per cent require split dosage. The second dose may be given presupper or at bedtime alone or as a mixture. Premeal and bedtime tests are used for regulation, the objective being to keep these sugar-free or nearly so.

On days of illness the maintenance dose is supplemented with regular insulin at 3-hour to 4-hour intervals according to test.

The dietary prescription is planned to aid in control and also promote growth. A useful caloric prescription is 1,000 calories at age 1, 100 calories added per year of age until the completion of growth and development. Then adult prescriptions of 15 calories per pound of ideal body weight may be used.

The partition of the diet into its component parts, carbohydrate, protein, and fat, may be normal (50 per cent—15 per cent—35 per cent) or the reverse, or such a variation as 40—20—40, which is our choice. Individualization, of course, is necessary.

Rapid desugarization is aided by overinsulinization and undernutrition.

Attempts to Alter the Course and Outcome

In addition to the use of insulins and dietary regulation to alter the course and outcome of juvenile diabetes, additional attempts have been made, as follows:

1. *Overinsulinization to produce and maintain the remission phase,* although supported on theoretical grounds by the experiments of Best and of Lukens, has not succeeded permanently. This scheme was advocated for children first by Brush.

2. The use of sulfonylureas (11) to inhibit insulinase or to produce islet hyperplasia has not been successful in juveniles (Chap. 35).

3. The preservation of islet tissue and function by cortisone, estrogen, thyroxin, hydrocortisone, shown conclusively in experimental diabetes by Houssay, Foglia, and Rodrigues has been attempted with some degree of success in juveniles. Among long-term juvenile diabetics treated with estrogen and progesterone in pregnancy, 50 per cent showed a permanent 50 per cent decrease in insulin requirement, and 20 per cent showed a permanent 75 per cent decrease. (They did not respond to sulfonylureas and insulin-like activity of the blood has not been determined.) Among 25 juveniles with newly contracted diabetes treated with insulin, dietary regulation, and subdiabetogenic doses of cortisone, one showed a response atypical for the natural course of diabetes. In the sixth year of diabetes, at age 18, he requires no insulin, achieves perfect control with 1 gm. of Orinase daily.

4. The fourth attempt to modify the outcome of juvenile diabetes has been with the use of biguanide (phenethylamidinyliminourea). In the past 18 months (January 1, 1957, to June 1, 1958) 100 patients with onset under age 18 have received this drug. Because it is new therapy, these cases are reported in some detail here. From the original 100, 10 are excluded: for lack of proof of diabetes in 1, for lack of co-operation in 3, and lack of conclusive data in 6. Eighteen additional patients received doses suboptimal for the hypoglycemic effect. The purpose of their therapy was restoration of dilated venules observed in the bulbar conjunctiva. Seventy-two cases are left for therapeutic evaluation.

The clinical characteristics of the patients varied. The sex distribution was even. The range for duration of diabetes was 0.1 to 35 years, and the average duration was 9 years. The age at the time of study varied from 4 to 46 years, the average being 16.5 years. The insulin requirement varied from 4 to 100 units at the time of the therapeutic trial and the average was 40 units. Thus, the typical patient was an adolescent of 16 years of age with well-established diabetes of 9 years' duration, requiring 40 units of insulin daily.

In addition to the evaluation of the hypoglycemic action, toxicity, and side effects, the value of these biguanides as a practical part of therapy in juvenile patients, their effect upon physiological and chemical control, upon the course and outcome of diabetes, and techniques of desugarization were attempted.

The desired hypoglycemic action alone or in combination with insulin was obtained in 64, or 89 per cent. Twenty-five per cent achieved this without insulin. In 8, or 11 per cent, the hypoglycemic effect was not demonstrated in the dose level used. The desired hypoglycemic action

is defined as normoglycemia, found fasting at 7:00 A.M., prelunch at 11:00 A.M., and presupper at 3:00 P.M.

In spite of the favorable hypoglycemic effect with currently available forms of biguanide, 29 of the 64 responsive cases spontaneously or on advice omitted the drug because of side effects, including nausea, lassitude, dry mouth, vomiting, or diarrhea.

The 30 who are still maintained on biguanide (DBI) show a significant response. The average dose of insulin is 10 units, and that of DBI 152 mg. At the time, the average age was 14, duration 6 years. The average dose of insulin at the onset of therapy was 28 units. Using the response to sulfonylureas as the basis on which 90 per cent respond to the drug in the first 6 months, 60 per cent from 6 to 12 months' duration, 40 per cent 1 to 2 years, 30 per cent 2 to 3 years, and 6 per cent after 5 years, these patients have done better than expected.

In the 18 months of use, no juvenile has developed ketoacidosis. In fact, the CO_2 rises on the average to 30 volumes per cent. Accidental omission of the drug, dietary indiscretions, and infections were followed by hyperglycemia and glycosuria and are corrected with increase in the dose of biguanide.

After initial stabilization significant hypoglycemia (below 60 mg.) was rare. None had unconsciousness or convulsions or even confusion.

Physiological control paralleled chemical control. Linear growth was satisfactory, even in patients receiving no insulin. The increment averaged 0.4 inch for 2 months, 0.8 inch for 6 months, and 1.5 inches for 12 months. The gain in weight was satisfactory, averaging 3.5 pounds for 4 months and 9.5 pounds for 12 months

The 14 cases remaining on DBI alone (from 1 to 18 months) are doing better than anticipated, since the remission phase of possible treatment without insulin is usually lost in 3 to 12 months.

The effect upon the prevention of vascular damage cannot be predicted, but the degree of restoration toward normal venular tone was greater with DBI or DBI plus insulin than with insulin alone.

No toxicity was demonstrable in tests for liver function, red and white blood counts, and hemoglobin values. But the side effects were numerous, including anorexia 39, nausea 29, vomiting 12, lassitude or drowsiness 3, diarrhea 3.

TECHNIQUE: DOSAGE. The time-action curve of biguanides resembles that of regular insulin but the hypoglycemic effect is slow, 4 hours, and duration 8 hours. The proposed dose schedule per pound of body weight is 0.6 mg. The upper limit is 200 mg Side effects may be reduced by a gradual slow transfer to the drug. Meticulous dietary control remains essential.

PHYSIOLOGY. The site of action is unknown, possibly in the citric acid cycle. Its clinical action appears to be that of potentiation of the action of either exogenous or endogenous insulin.

PSYCHOLOGY. The effect of the potential use of this oral substitute with the implication of newer discoveries upon parents, patients, and physicians is overwhelming.

5. The fifth attempt to alter the course of diabetes has been grafting the fetal pancreas of the neonatally dying infant of the former juvenile diabetic. The transplants include the whole pancreas once, fragmented pancreatic tissue three times, and inclusion in a milipore chamber once. The material is ideal, β-cells almost exclusively and no digestive ferments, but immune mechanisms still block success. Not discouraged with these failures, new conceptions for transplants are being developed.

The prognosis of juvenile diabetes, measured by years of duration, is limited by the span of the insulin era. Among 4,054 juveniles, 5 per cent have survived 35 years, 18 per cent 25 years. The estimated life span is three fourth of the normal. The chief cause of death is diabetic nephropathy.

Conclusions

The tendency to develop diabetes in childhood is inherited. The disease follows a significant natural course, acute onset, virulent early course, remission, intensification to total diabetes. The problems of growth and development have resolved themselves. The control of coma and sepsis is successful. Nephropathy has replaced other causes of death. To vascular disease poor chemical control remains an important contributing factor. A predisposition is also suggested in prediabetes. Present forms of available insulin, along with dietary regulation, supplemented by a new oral hypoglycemic agent should facilitate control, and other new programs to alter the course of diabetes are in progress.

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Chapter 30

DIAGNOSTIC TESTS FOR DIABETES MELLITUS

Stefan S. Fajans

INTRODUCTION

The recognition of diabetes mellitus depends upon the use and interpretation of the proper laboratory procedures. When classic symptoms or complications of diabetes are present, the diagnosis is usually suspected and presents no problem. However, in the early or mild forms of the disease, when the patient is asymptomatic, the diagnosis may be delayed for many years or missed unless laboratory tests are freely employed. In some cases, the presence of latent diabetes will be recognized by routine performance of urinalysis and determination of a fasting blood sugar level. In the mildest form of the disease, these procedures may be of little aid. Here, the only recognizable abnormality will be a diminished ability to utilize a carbohydrate load, as demonstrated by a glucose tolerance test.

The early detection of diabetes is vital. Easier control of the disease is facilitated, and progression of mild to frank diabetes may be prevented. If any measures are to be effective in retarding or preventing the occurrence or severity of complications of diabetes, they will have to be applied early in the course of the disease. Indeed, prevention of diabetes

and its complications may hinge on the recognition of a diabetic diathesis not discernible by tests in routine use today. With these aims in mind, tests for the recognition of potential or future diabetes are the subject of current investigations.

Accordingly, it is the purpose of this chapter to discuss: (1) the methods and criteria used to establish a diagnosis of diabetes mellitus, (2) screening procedures employed for the early detection of diabetes mellitus, and (3) tests used for the possible prediction of future diabetes mellitus. In addition, (4) ancillary procedures used in the evaluation of abnormalities of carbohydrate metabolism, (5) meliturias, and (6) tests used for detection of urinary and plasma ketone bodies are presented.

METHODS AND CRITERIA FOR DIAGNOSIS OF *DIABETES MELLITUS*

GLUCOSURIA

The finding of glucosuria should always suggest the existence of diabetes mellitus. Usually glucosuria is the result of hyperglycemia, glucose appearing in the urine when the blood sugar level exceeds the renal threshold.* In moderate or severe grades of diabetes mellitus, glucosuria will be continuous and present even after an overnight fast. In mild forms of the disease it may appear only within two hours after a high carbohydrate meal.

The presence of glucosuria never establishes the diagnosis, and blood sugar determinations must be made to confirm or eliminate the diagnosis of diabetes. Renal and alimentary glucosuria must be differentiated from diabetic glucosuria as discussed later. Other meliturias and nonsugar reducing substances in urine which may give false positive reactions for glucose also must be considered.

On the other hand, the absence of glucosuria does not eliminate the possibility of the presence of diabetes mellitus. Even postprandial glucosuria may not appear in the mildest form of the disease when the diagnosis can be made by performance of the glucose tolerance test only. Glucosuria may also be absent even in the presence of more definite hyperglycemia if the renal threshold for glucose is elevated.

Tests for Glucosuria

METALLO-REDUCTION TESTS. The following tests are based on reduction of alkaline copper or bismuth reagents.

Benedict's Solution. The solution contains copper sulfate, sodium bi-

* "Renal threshold" is discussed in section on nondiabetic meliturria

carbonate, and sodium citrate. Glucose, when boiled with this alkaline copper solution, causes formation of cuprous oxide, changing the color of the solution. This test is used for qualitative detection of glucose as well as for approximation of severity of glucosuria.

Directions: To 5 ml. of Benedict's qualitative solution, add 8 drops of urine in a test tube and shake. The tube is placed in a boiling water bath and boiled for three minutes. If the color of the solution in the tube is unchanged at the end of that time the test is negative. (Boiling longer than three minutes may cause the solution to turn a blue-green color, which does not necessarily indicate that sugar is present.) If at the end of three minutes of boiling the solution has changed color, continue boiling for an additional two minutes and record the color. A clear green color is read as 1+ (less than 0.5 per cent glucose), a green with yellow sediment as 2+ (0.5–1.0 per cent), a yellow-orange color as 3+ (1.0–2.0 per cent), and a brick-red color indicates 4+ glucosuria (over 2 per cent).

Benedict's quantitative test for sugar in urine is used to ascertain the total amount of glucose excreted in 24 hours. The technique is described in any standard text on laboratory procedure.

Clinitest. The Clinitest reagent tablet contains anhydrous copper sulfate, anhydrous sodium hydroxide, citric acid, and sodium bicarbonate. The test is made by placing 5 drops of urine in a test tube, then adding 10 drops of water and a reagent tablet. The solution will boil with the heat generated by the chemical reaction. Fifteen seconds after the boiling has stopped the tube is shaken and the color compared with the color scale. A trace of sugar (0.25 per cent) will cause the solution to turn dark green, 1+ (0 5 per cent) grass green, 2+ (0.75 per cent) greenish-brown, 3+ (1 per cent) tan, and 4+ (2 per cent) orange. If the color change should pass through orange and to a dark greenish-brown while the solution boils the concentration is recorded as over 2 per cent. The reagent tablets are very hydroscopic. Tablets which have absorbed moisture turn blue and should not be used in testing.

Galatest. The Galatest reagent contains bismuth oxychloride, sodium hydroxide, and sodium silicate. A small amount of the reagent is placed on a piece of white paper. One drop of urine is added and after 30 seconds the resultant color is compared with the standard color scale, which ranges from gray to black.

The above methods for the determination of glucose in urine are well established and useful. However, the methods lack specificity since they will detect reducing substances other than glucose. False positive reactions for glucose appear in the presence of other sugars such as fructose, lactose, galactose, pentoses, and in the presence of large

amounts of conjugated glucuronates which may appear after the ingestion of salicylates, aminopyrine, paraminobenzoic acid, chloral hydrate, camphor, and menthol. Homogentisic acid and large amounts of ascorbic acid, uric acid, or creatinine will also give false positive reactions.

SPECIFIC METHODS FOR GLUCOSE. A relatively simple quantitative method for the specific determination of glucose in urine and blood using the enzyme glucose oxidase has recently become available. Specificity results from the exclusive oxidation of beta-glucose to gluconic acid by this enzyme. The method is especially suitable for the accurate determination of small amounts of glucose in urine. This enzymatic method has also been used in developing sensitive and simple absorbent indicator papers for the specific and rapid detection of glucose in urine. The indicator papers are impregnated with glucose oxidase, peroxidase, and orthotolidine. Glucose oxidase reacts with beta-glucose and atmospheric oxygen to produce gluconic acid and hydrogen peroxide. In the presence of peroxidase the hydrogen peroxide formed oxidizes orthotolidine, which turns the paper blue.

One disadvantage of the routine use of enzyme papers for urinalysis is the fact that other nonglucose reducing sugars detected by the copper-reducing methods will be missed.

Tes-Tape. Tes-Tape is one of the indicators that has been used for detection as well as for estimation of the amount of glucosuria. Formerly it appeared that the color response often failed to distinguish between moderate and profound degrees of glucosuria. However, with a technically improved Tes-Tape and revised recommendations for its use it appears that Tes-Tape may provide the desirable combination of convenience and reliability in the semiquantitative estimation of urinary glucose.

Directions:

1. A 1½-inch strip of Tes-Tape is dipped into a sample of urine and laid against a white background. Saturation of the paper strip is such that the fluid front advances slowly, but that a dry portion always remains.

2. After one minute of color development the reading is made by comparing the area of most intense blue-green hue with the color chart on the Tes-Tape container.

3. If the one minute reading is 0.5 per cent (3+) or more a final reading should be made after 2 minutes of color development for greater accuracy.

Clinistix. Clinistix is another glucose-oxidase impregnated test paper for the specific detection of glucose in urine. The test paper is dipped

into urine and observed at exactly one minute. When sugar is present the wetted strip turns blue. The minimum concentration of glucose detectable ranges from 0.01 to 0.1 per cent.

Uristix. Uristix is an impregnated test paper for the detection of both glucosuria and proteinuria. The paper strip is dipped into urine, and the second portion of the indicator strip is compared with the color chart for glucose after 10 seconds. The tip of the paper is compared with the color chart for protein thereafter.

OTHER METHODS FOR URINARY GLUCOSE. Since the availability of a specific enzymatic (glucose oxidase) method, other tests for the identification of glucose in urine such as fermentation tests, formation of osazones with phenylhydrazine, and polaroscopic light tests are rarely used clinically. However, these techniques are still useful aids in the identification of other nonglucose reducing sugars such as fructose, lactose, galactose and pentoses. Paper chromatography has also been used to identify various sugars which occur in urine.

FASTING BLOOD SUGAR

The fasting blood sugar level is elevated above normal in patients with moderately severe or severe diabetes. A definite elevation establishes the diagnosis. Patients with mild diabetes, however, may have a fasting blood sugar level within normal limits. In such patients either a blood sugar obtained after a high carbohydrate meal (postprandial blood sugar) or a glucose tolerance test will indicate the presence of diabetes.

The range of normal values for the fasting blood sugar level in venous blood depends upon the method of analysis employed. Normal values as determined by a "true blood sugar method," such as the Somogyi-Nelson method, range between 60 and 100 mg. per 100 ml. Values above 120 mg. per 100 ml., if confirmed by analysis of a second sample, may be regarded as diagnostic of diabetes mellitus. Values between 100 and 120 mg. per 100 ml., although abnormal, should not be accepted as diagnostic of diabetes unless supported by evidence of diminished carbohydrate tolerance. Transient elevation of the fasting blood sugar may occur in the nondiabetic owing to increased release of epinephrine in the frightened, excited, or apprehensive patient. The author has not observed a fasting blood sugar value above 108 mg. per 100 ml. in an individual with an otherwise normal glucose tolerance test. All figures used in this chapter, unless otherwise stated, are "true blood sugar" values, which exclude nonglucose reducing substances.

Probably the most widely used blood sugar method is the older Folin-Wu procedure which measures, in addition to glucose, nonglucose

reducing substances such as ergothioneine and glutathione. These substances [illegible] ml. of bloo[illegible] ng. per cer[illegible] rue blood sugar) method.

Capillary blood sugar values obtained after an overnight fast are only 2 or 3 mg. higher than those in venous blood. Normal values depend on the method of analysis.

CARBOHYDRATE TOLERANCE TESTS

Diminished ability to utilize a carbohydrate load with excessive postprandial hyperglycemia and a delayed rate of return of the blood sugar level to normal characterizes the metabolic defect in diabetes. In a patient with a normal or mildly elevated fasting blood sugar level, a carbohydrate tolerance test must be performed to establish or eliminate the diagnosis of diabetes mellitus. The test is also used to distinguish diabetic from nondiabetic glucosuria. Though the glucose tolerance test lacks specificity—not every high prolonged curve being indicative of diabetes mellitus—it nevertheless is the most sensitive diagnostic procedure. However, the test is unnecessary if the level of fasting blood sugar is diagnostic: above 120 mg. per ml. by a true blood sugar method.

In general, three main types of glucose tolerance tests have been used. These are the standard oral glucose tolerance test employing a single dose of glucose, the two-dose oral glucose tolerance test, and the intravenous glucose tolerance test. Various modifications of the oral single dose as well as of the intravenous glucose tolerance test have been employed.

When the glucose is given orally, its absorption from the gastrointestinal tract into the blood continues for a variable period of time, depending upon the amount of glucose given. Maximum absorption of glucose has been found to be 0.8 gm. per kilogram body weight per hour. The oral glucose tolerance test measures the balance between the rate of passage of glucose into extracellular fluid and its removal by cellular assimilation (and urinary excretion, if any). Thus, the test will be influenced not only by variables affecting utilization of glucose but also by factors that influence its absorption.

It has been suggested that the over-all removal rate for orally administered glucose is considerably above that for glucose administered intravenously and that the entry of glucose into the circulation via the portal route results in its greater hepatic disposal than after its introduction into the systemic circulation. Thus, although the intravenous glucose tolerance test eliminates the factor of absorption, the standard

oral glucose tolerance test may be considered to be a more physiological test and to give a better index of total carbohydrate utilization.

Standard Oral Glucose Tolerance Test (Single Dose)

OGTT

METHOD. For 3 days preceding the glucose tolerance test the patient is advised to ingest a diet containing approximately 300 gm. of carbohydrate and maintenance calories (Conn). If there has been severe dietary limitation of carbohydrate or calories, or if recent weight loss has occurred, ingestion of this diet is advised for 5 days.

Although ingestion of smaller amounts of carbohydrate (150 gm. or more) is probably sufficient to prevent plateau or diabetic-type curves in normal subjects, a standardized high carbohydrate preparation diet is advised to ensure reproducibility of the test. In mild diabetics a low carbohydrate intake may improve an abnormal glucose tolerance curve to such an extent that it will become nondiagnostic. An outline of the standard preparatory diet containing 300 gm. of carbohydrate and 3000 calories (Table 30-1) is given to each patient scheduled for a glucose tolerance test. If 3000 calories are greatly in excess of maintenance calories the patient is advised to decrease the calories derived from fat. The overnight fast should be at least 9½ hours before beginning the test. The loading dose of glucose used is, depending on preference, either 100 gm. of glucose or 1.75 gm. of glucose per kilogram of ideal body weight calculated from the subject's height and age. The glucose is dissolved in 400 cc. of water, flavored with lemon juice or lemon extract, and chilled. Venous blood is obtained before and every ½ hour or every hour for 3 hours after the ingestion of glucose. A urine specimen is obtained with each blood specimen. If a history of symptoms of reactive hypoglycemia three or more hours after a meal can be obtained the test is prolonged to four hours.

INTERPRETATION. The criteria used for the interpretation of the glucose tolerance test are as follows:

Normal Test. Carbohydrate tolerance is considered to be normal when the oral glucose tolerance test gives the following results (Table 30-2): a fasting blood sugar level less than 100 mg. per 100 ml., a peak value of less than 160 mg per 100 ml., and a 2-hour value of less than 110 mg. per 100 ml A mean curve derived from the results of glucose tolerance tests performed in 125 nondiabetic subjects without a family history of diabetes is shown in Figure 30-1 The mean peak blood sugar level is 129 mg /100 ml at ½ hour and the mean blood level 2 hours after ingestion of glucose is 87 mg./100 ml. Flat glucose tolerance curves are seen in many hea[illegible] suggest malabsorption of glucose [illegible] uch as sprue

TABLE 30-1. GLUCOSE TOLERANCE PREPARATION DIET*

The diet contains approximately the following:
Protein: 80 Gm. Carbohydrate: 300 Gm. Calories: 3000

Breakfast	
Fruit or fruit juice, fresh or cooked	1 serving
Cereal	½ cup cooked or ¾ cup prepared
Bread	2 slices
Butter or oleomargerine	1 tablespoon (2 pats) or 3 strips bacon
Cream	½ cup
Jelly or sugar	2 tablespoons
Dinner	
Meat, fish, eggs or cheese	1 average serving
Potato, rice or macaroni	1 average serving
Cooked vegetable or salad	1 serving
Bread	2 slices
Dessert—pie, cake, pudding, ice cream, or fruit with cookies	1 average serving
Cream	3 tablespoons
Jelly or sugar	2 tablespoons
Butter or oleomargerine	2 tablespoons

(A packed lunch may consist of two (2) meat, cheese, or egg sandwiches, fruit, cake or cookies, and a candy bar.)

Supper	
Meat, fish, eggs, or cheese	1 average serving
Potato, rice, or macaroni	1 average serving
Cooked vegetable or salad	1 serving
Bread	2 slices
Dessert: pie, cake, pudding, ice cream, or fruit with cookies	1 average serving
Jelly or sugar	2 tablespoons
Cream	3 tablespoons
Butter or oleomargarine	2 tablespoons

One pint (2 cups) of milk is to be used during the day. Coffee and tea may be used as desired.

In case all the butter, cream, sugar, and jelly cannot be eaten conveniently at any meal, a two (2) ounce chocolate nut bar may be substituted. However, it is better if *the entire diet as stated can be eaten.*

No food or drink is to be taken after 10:30 the night before the test.

* Nutrition Clinic, University of Michigan Medical Center.

or pylorospasm. Mild variations of glucose tolerance may be encountered when the test is repeated at intervals of more than a few days in the same individual. In general, however, there is a high degree of reproducibility when the oral glucose tolerance test is carefully performed in conjunction with the use of the standard high carbohydrate preparatory diet and a true blood sugar method.

TABLE 30-2. CRITERIA FOR INTERPRETATION OF GLUCOSE TOLERANCE TEST
(Venous Blood and True Blood Sugar Method*)

Time Hours	*Normal*	*Diabetes*	*Probable diabetes*
F	Less than 100 mg./100 ml.		
½	Less than 160 mg./100 ml.		
1	Less than 160 mg./100 ml.	160 mg./100 ml. or more	160 mg./100 ml. or more
1½		140 mg./100 ml. or more	135 mg./100 ml. or more
2	Less than 110 mg./100 ml.	120 mg./100 ml. or more	110 mg./100 ml. or more

* Somogyi-Nelson method.

Tests Indicating Diabetes Mellitus. The criteria employed for the interpretation of an abnormal oral glucose tolerance test vary within relatively narrow limits in various clinics. In the absence of other disease, gross abnormalities of glucose tolerance are considered diagnostic of diabetes by everyone. The general characteristics of glucose tolerance tests indicating diabetes are a rise of the blood sugar to an excessively high peak level and a failure of the level to return to normal *two hours* after ingestion of glucose. The delayed return to normal is the most characteristic feature of a diabetic test. The time of maximum concentration of blood sugar is variable: the greater the rise the later the peak level is attained.

There is still considerable difference of opinion as to the interpretation of mild abnormalities of glucose tolerance to be considered as diagnostic of diabetes mellitus. The following criteria, although strict, seem sound when employed in the ambulatory and otherwise healthy patient. They do not apply to patients confined to bed, or suffering from acute or severe chronic illness.

The combination of the one-hour value of 160 mg./100 ml. or above plus a two-hour value of 120 mg. or above is regarded as diagnostic of

the diabetic state. In borderline curves the level at 1½ hours is required to be 140 mg./100 ml. or above to be diagnostic (Table 30-2). This eliminates a false diagnosis of diabetes on the occasional curve which drops abruptly to normal at 1½ hours and rebounds above 120 mg. per cent by 2 hours. Figure 30-1 illustrates these criteria. A diabetic curve

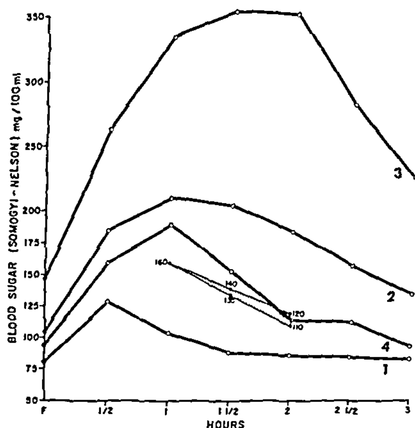


FIG. 30-1. Glucose tolerance tests. Shaded triangle illustrates diagnostic criteria discussed in text. Curve 1, mean curve from 125 nondiabetic control subjects, Curve 2, diabetes, Curve 3, diabetes; glucose tolerance test unnecessary as fasting blood sugar diagnostic (147 mg./100 ml.); Curve 4, probable diabetes.

is one in which all points lie at or above the upper border of the shaded triangle (Fajans and Conn). The following is an example of a rebound curve that should not be considered diagnostic even though the two-hour level is elevated:

F	½	1	1½	2	2½	3
88	152	162	98	125	106	68

When the one-hour value exceeds 160 mg. per cent/100 ml. and the two-hour value falls between 110 and 120 mg./100 ml. a diagnosis of "probable diabetes" is made. Again, to eliminate rebound curves the level at 1½ hours is required to be 135 mg./100 ml. or above to be diagnostic of probable diabetes (Table 30-2). In Figure 30-1 a curve indicating probable diabetes is one which lies at or above the lower border of the shaded zone.

Terminal hypoglycemia 3 to 5 hours after ingestion of glucose occurs in a large number of patients whose glucose tolerance tests disclose the presence of mild diabetes mellitus. Many of these patients have symptoms of spontaneous hypoglycemia suggestive of those seen in functional hyperinsulinism. Forty-four per cent of 110 patients with glucose tolerance tests that exhibited early hyperglycemia and late hypoglycemia had [illegible]
hour [illegible]
may [illegible]

It [illegible]
above are arbitrary, and that judgment has to be exercised in the interpretation of the glucose tolerance test when blood sugar values deviate by only a few milligrams per 100 ml. above or below these figures. There are some investigators and practicing physicians who will regard these criteria as insufficient evidence on which to base a diagnosis of diabetes mellitus or probable diabetes mellitus. Using these criteria, Fajans and Conn found only one glucose tolerance test indicating diabetes and one indicating probable diabetes among 127 healthy subjects without a family history of diabetes mellitus. Also, in line with current emphasis upon the discovery and control of early mild diabetes, it is most important not to ignore mild abnormalities of glucose tolerance. Careful follow-up of patients with such tests frequently shows further impairment of carbohydrate utilization (Table 30-3). In middle-aged individuals loss of glucose tolerance may be only slowly progressive over many years. In young patients decompensation of the diabetes with development of fasting hyperglycemia may occur rapidly. Jackson has also stressed the significance of minor aberrations of glucose tolerance. He has reported patients showing mildly abnormal or borderline curves who have later developed clearly diagnostic abnormalities.

The criteria described above are similar to those of Mosenthal and Barry, and Moyer and Womack. Mosenthal, employing the true blood sugar method on venous blood in ambulant individuals established the following somewhat more restrictive criteria as indicating diminished carbohydrate tolerance: Maximum value of the blood sugar level above

150 mg./100 ml. and a level above 100 mg./100 ml. two hours after *ingestion of glucose.*

Lukens quotes the following diagnostic criteria. A rating of 1 is given to each of the following: (1) a fasting blood sugar value above 110 mg./100 ml., (2) elevations of blood sugar levels above 170 mg./100 ml. at 1 hour and above 120 mg./100 ml. at 2 hours, and (3) a blood sugar level above 110 mg. /100 ml. at 3 hours. If any two of the three ratings are abnormal, the patient is considered to be diabetic.

TABLE 30-3. PROGNOSTIC IMPORTANCE OF MILD ABNORMALITIES OF GLUCOSE TOLERANCE

				Glucose tolerance test			
Subject and height	*Year*	*Age*	*Weight*	*Fasting*	*1 hr.*	*2 hr.*	*3 hr.*
				0	++	++++	++
L. H., male, 6'2"	1939	59	240	83	186	122	58
	1950	70	227	134	262	301	205
				+	+++	++++	++++
A. W., male, 5'7"	1934	46	119	81	170	115	89
				0	++	++++	+
	1936	48	149	86	166	150	100
				+	+++	++++	+++
	1956	68	150½	84	201	241	151

However, since it is agreed by everyone that diabetes mellitus may exist without an elevation of the fasting blood sugar, the fasting level should *not* be one of the criteria for interpretation of an abnormal glucose tolerance test. Also, the requirement of an elevation of the blood sugar three hours after ingestion of glucose as part of a set of diagnostic criteria would eliminate the diagnosis of diabetes mellitus or probable diabetes in many patients in whom the diagnosis is made by the criteria of abnormal peak and two-hour elevation only. Repeated tests over a period of time in these patients illustrate progression to unquestionable diabetes mellitus (Table 30-3).

Fasting and Two-Hour Levels Only. In order to save multiple venopunctures, time, effort, and expense, an abbreviated glucose tolerance test is performed by some by obtaining only the fasting blood sugar and the level two hours after the ingestion of glucose. This practice decreases the sensitivity of the test. Elevation of the two-hour blood sugar level above 140 mg./100 ml. can be considered as diagnostic of diabetes mellitus but lower values are not. Rebound curves (see page 398) and other unusual curves do occur. Therefore, the presence of diabetes or

probable diabetes cannot be established by a single two-hour blood sugar value between 110 and 140 mg./100 ml., even though values in this range are most suggestive of this diagnosis. The following unusual curve obtained in a healthy 20-year old subject has to be considered within normal limits even though the two-hour level is elevated.

F	½	1	1½	2	2½	3
74	108	107	108	127	107	92

Abnormally High Peak Level Only. A diagnosis of diabetes mellitus cannot be made with confidence on the basis of an abnormal elevation of the blood sugar level (above 160 mg. per cent) at ½ or 1 hour of the glucose tolerance test if it is accompanied by a normal two-hour blood sugar level. Rapid progression of glucose through the pylorus may give rise to abnormally rapid intestinal absorption of glucose and to high peak values of the blood sugar. This occurs in patients with gastroenterostomies or subtotal gastrectomies. However, an abnormally high peak blood sugar level in an otherwise healthy individual should be regarded with suspicion and careful follow-up of the patient planned. Rapid transition of such curves to curves diagnostic for diabetes mellitus occur as illustrated in Table 30-4.

TABLE 30-4. PROGNOSTIC IMPORTANCE OF ABNORMALLY HIGH PEAK BLOOD SUGAR LEVELS WITH NORMAL TWO-HOUR LEVELS

				Glucose tolerance test						
Subject and height	*Date*	*Age*	*Weight*	*Fasting*	*½ hr*	*1 hr*	*1½ hr*	*2 hr*	*2½ hr*	*3 hr*
M. P., male, 6′	1935	43	160	0 103		++++ 174		++++ 93		0 68
	1940	48	161	++++ 211						
A. M., female, 5′5″	3-5-53	33	160	0 100		++ 180		+ 96		+ 118
	6-25-53	33	162	123	214	259	189	158	163	137
J G., male, 5′10″	3-15-55	24	178	0 98	+++ 198	+++ 153		+++ 76		0 56
	6-1-55	24	177	0 88	188	+++ 207	+++ 214	+++ 218	143	+++ 140
C. R., male, 5′8″	1-11-57	24	178	0 91	++ 176	+++ 158		100		62
	3-21-57	24	178	92	139	193	176	154	138	138
	6-23-58	25	156	89	177	203	187	151	123	129

Reversibility of Abnormal Glucose Tolerance Test. It has been recognized for many years that reduction of body weight in the obese middle-aged diabetic may result in the return to normal not only of

the elevated fasting blood sugar but also of the abnormal glucose tolerance test. It is less well recognized that spontaneous reversals of abnormal glucose tolerance tests to normal may also occur in young diabetics. Thus, a change from a glucose tolerance test diagnostic of diabetes by the criteria listed to a normal one does not invalidate per se these criteria. An extreme example of such a change to a nondiagnostic glucose tolerance test with subsequent development of severe diabetes with complications is given in Table 30-5.

TABLE 30-5. REVERSIBILITY OF DIAGNOSTIC GLUCOSE TOLERANCE TEST

B. S., Male
Height 5'6", Wt 152 pounds — 3-16-38 Glucosuria found; no symptoms

		Glucose tolerance test			
Date	*Age*	*Fasting*	*1 hr.*	*2 hr.*	*3 hr.*
		0	++++	++++	+++
3-18-38	21	211	375	353	273
		0	+	+	+
3-29-38	21	107	146	112	99
1946	32	Furunculosis, polydipsea, polyphagia, 8 lb wt loss; then recovered without treatment			
1947	33	Abdominal wall abscess, fatigue: *FBS. 550* mg /*100* ml Diagnosis: Diabetes mellitus Diet and insulin			
1948	34	Retinitis proliferans, vitreous hemorrhages			

Use of Folin-Wu Blood Sugar Method. As emphasized above, the criteria given for the interpretation of the glucose tolerance test are for use with a true blood sugar method. If the Folin-Wu blood sugar method is employed, critical values are usually set 20 mg. per 100 ml. higher. However, it has been shown that the Folin-Wu technique includes frequently unpredictable amounts of nonglucose reducing substances varying from less than 10 to 80 mg./100 ml. Mosenthal found that in 27 per cent of blood sugar determinations the values for nonglucose reducing substances exceeded 30 mg./100 ml. For practical purposes the Folin-Wu technique is adequate when normal levels or definite elevations of blood sugar values above normal (50 mg./100 ml. or more) are

found. However, with levels in the borderline range the Folin-Wu technique may give misleading results.

Use of Capillary Blood. There is a minimal difference between the level of *fasting* blood sugar in venous and arterial or capillary blood. After a carbohydrate load the concentration of glucose in capillary blood is from 20 to 70 mg./100 ml. higher than in venous blood in normal subjects. This difference is due to utilization of glucose by the tissues. A capillary blood sugar level below 130 mg./100 ml. at two hours using the true blood sugar method may be within normal limits. Performance of the glucose tolerance test with the use of capillary blood is less satisfactory since there are fluctuations in the capillary-venous blood sugar differences. Considerable experience, skill, and care are required to obtain capillary blood satisfactory for analysis.

INDICATIONS FOR GLUCOSE TOLERANCE TEST. Performance of the glucose tolerance test is indicated when the diagnosis of diabetes cannot be made or excluded without its use. Since the glucose tolerance test is the most sensitive of the presently established tests for the diagnosis of diabetes mellitus, its use is advocated as a routine procedure in individuals in whom the disease may exist in the latent or subclinical stage. Such people will fall into one or more of the following groups:

1. Patients with glucosuria without diagnostic hyperglycemia after an overnight fast.
2. Patients with otherwise unexplained neuropathy, retinopathy, nephropathy, peripheral vascular disease, or coronary artery disease. Not infrequently one of the "complications" of diabetes mellitus may actually lead to its diagnosis.
3. A family history of diabetes. Among 400 apparently healthy relatives (siblings, children, or parents) of diabetic patients a 19 per cent incidence of diabetes and a 3.5 per cent incidence of "probable diabetes" has been found. Individuals who are obese and middle-aged should be particular candidates for routine testing.
4. Patients with a history of previous pregnancies productive of abortions, premature labor, stillbirths (particularly if islet cell hypertrophy of the pancreas is found), neonatal deaths, or large babies (over 9 pounds). Glucosuria found for the first time during pregnancy should not be assumed to be due to a lowering of the renal threshold. A glucose tolerance test should be performed during pregnancy rather than await the postpartum period (see below).
5. Individuals who were large babies themselves.
6. Patients with transitory glucosuria or nondiagnostic hyperglycemia found in the course of surgical procedures, trauma, emotional stress, myocardial infarction, cerebrovascular accident, or administration of

adrenal steroids. The test should be performed after recovery from acute stress or illness. Glucosuria is important even if found in only one examination. The probability of subsequent development of frank diabetes in such cases has been found to be very high.

7. Patients with renal or alimentary glucosuria should be tested periodically for the development of diabetes. See section on nondiabetic melituria.

8. Patients with symptoms of spontaneous hypoglycemia, particularly those with a family history of diabetes (see page 399).

Patients in the above categories with normal glucose tolerance tests should be tested at regular intervals through the years since a normal test does not rule out the development of diabetes mellitus in subsequent years.

CONDITIONS WHICH ALTER CARBOHYDRATE TOLERANCE. In normal and mildly diabetic subjects carbohydrate tolerance can be improved by vigorous exercise. Tests performed on successive days may also increase tolerance. Prolonged physical inactivity decreases tolerance. Some authors believe that glucose tolerance gradually decreases with age but this is at variance with reports from other workers.

Not every abnormal glucose tolerance test indicates the presence of diabetes mellitus. Plateau curves preceded by normal or low fasting blood sugar levels occur during starvation (starvation diabetes), in severe liver disease and glycogen storage disease. Prior insulin administration has been shown to impair carbohydrate tolerance in normal subjects. Patients with functioning tumors of the pancreatic islet cells may have plateau curves if not adequately prepared with a high carbohydrate diet. In such patients the finding of fasting hypoglycemia will differentiate this condition from diabetes mellitus. The strict criteria employed for the diagnosis of diabetes mellitus and "probable diabetes" do not apply to the acutely ill patient or to the patient confined to bed by chronic illness. Acute emotional disturbances may decrease glucose tolerance. Patients with injury to the hypothalamus due to cerebrovascular accidents, infections, or trauma may not only have loss of carbohydrate tolerance and glucosuria but also transitory fasting hyperglycemia. However, it is likely that the hyperglycemia and glucosuria first detected in many patients following cerebrovascular accidents may not be due to hypothalamic involvement but may be due to exacerbation of previously undetected and latent diabetes.

The majority of patients with acromegaly, Cushing's syndrome, and pheochromocytomas have fasting hyperglycemia or diabetic glucose tolerance tests. The abnormality of carbohydrate metabolism returns to normal in almost all cases after correction of the endocrine lesion.

Some patients with severe, long-standing acromegaly may have residual diabetes owing to permanent damage to the islet cells.

In patients with severe thyrotoxicosis the glucose tolerance test can be interpreted only after ingestion of a high carbohydrate, high caloric diet for at least 5 days. An abnormally high peak blood sugar level with a normal two-hour value is due to increased intestinal absorption of glucose. A two-hour blood sugar level above 120 mg./100 ml. is evidence for the presence of diabetes mellitus. The glucose tolerance test should be repeated after correction of thyrotoxicosis. Even if the glucose tolerance test has returned to normal, subclinical diabetes mellitus made manifest by the stress of thyrotoxicosis cannot be eliminated.

Pregnancy sometimes causes mild loss of carbohydrate tolerance. Two-hour levels may be higher during pregnancy, but the glucose tolerance curve stays within normal limits. An abnormal glucose tolerance test should be considered significant even though it may return to normal shortly after termination of pregnancy. These may be women with early diabetes in whom high blood steroid levels and other factors associated with pregnancy have uncovered decreased islet-cell reserve. The frequent occurrence in such women of a family history of diabetes, birth of large babies, abortions, stillbirths, neonatal deaths, and subsequent development of clear-cut diabetes strengthens this impression.

Asphyxia and anesthesia will also decrease glucose tolerance by interfering with the normal rate of glycolysis.

Two-Hour Postprandial Blood Sugar

The determination of a single blood sugar level two hours after a carbohydrate meal containing approximately 100 gm. of glucose (Table

TABLE 30-6. 100-GRAM CARBOHYDRATE MEAL
(Approximately 100 Gm. carbohydrate)

		Gm. carbohydrate
Cereal, potatoes, macaroni, rice, or noodles	1 serving	15
Bread	2 slices	30
Sugar or jelly	1 tablespoon	15
Milk	½ pint	12
Canned fruit, ice cream sundae, cake, or pie	1 serving	
or orange juice	1 glass	25
		97

Other foods as desired
Blood should be drawn 2 hours after beginning of breakfast or lunch Meal to be finished within 30 minutes

30-6) is sometimes used to establish the diagnosis of diabetes mellitus. A two-hour postprandial blood sugar level of 140 mg./100 ml. or above indicates the presence of diabetes mellitus, if other factors that decrease carbohydrate tolerance can be eliminated (see page 404). Blood sugar levels between 110 and 140 mg./100 ml. should be considered as probably abnormal, and the diagnosis of diabetes mellitus should be established or ruled out by the performance of the standard oral glucose tolerance test. The two-hour postprandial blood sugar is of great value as a screening procedure but cannot replace the glucose tolerance test for sensitivity and reliability in the diagnosis of mild diabetes mellitus. Occasionally a glucose tolerance test diagnostic for diabetes is found in a patient with a two-hour postprandial blood sugar below 110 mg./100 ml.

One Hour, Two-Dose Glucose Tolerance Test (Exton-Rose)

With this test the fasting blood sugar is obtained, and 50 gm. of glucose are given orally immediately thereafter and again ½ hour later. Blood sugars are obtained ½ hour after the first and second glucose loads. The test requires only three venopunctures and one hour for completion. However, there is little agreement as to what constitutes an abnormal response to this test. Since a one-hour test can give only a presumptive diagnosis of diabetes it is no longer considered useful. If a simple test is to be used a single blood sugar two hours after ingestion of 100 gm. of glucose is more useful.

Intravenous Glucose Tolerance Tests

The intravenous glucose tolerance test has been rarely employed for the routine diagnosis of diabetes mellitus except when disturbance of intestinal absorption is present or anticipated. The administration of glucose by the intravenous route allows an evaluation of the capacity of the body to deal with the hyperglycemia that follows the administration of glucose unaffected by intestinal absorption.

The intravenous glucose tolerance test can be used with variations in glucose loads and timing of blood samples.

One-half gm. of glucose per kilogram of ideal body weight may be infused over a period of 30 minutes as a 20 per cent solution or given rapidly as a 50 per cent solution during 2 to 4 minutes. Blood samples are obtained in the fasting state and at ½-hour intervals for 3 hours. The test is abnormal if the blood sugar fails to return to normal fasting levels within 90 to 120 minutes after administration of the glucose load. Although this test is satisfactory for diagnostic use it has been reported

to be a less sensitive indicator of mild abnormalities of carbohydrate tolerance than the standard oral glucose tolerance test.

A rapid intravenous glucose tolerance test has also been used. Twenty-five grams of glucose are given intravenously over 3 to 4 minutes and samples of blood are obtained every 4 to 10 minutes for 60 to 90 minutes. This test has the advantage that the rate of disappearance of glucose from blood can be analyzed mathematically with calculation of an index (K) of glucose utilization. One index used expresses the rate of fall of the glucose level as a percentage of total blood sugar value ("glucose assimilation coefficient" or "total index") while the other expresses the rate as a percentage of the level in excess of the initial fasting value ("increment index").

SODIUM TOLBUTAMIDE RESPONSE TEST

The magnitude of the hypoglycemic response to intravenously administered sodium tolbutamide has been used to differentiate between nondiabetic and diabetic individuals. It has been reported that the test correctly separates mildly diabetic from nondiabetic patients in approximately 95 per cent of cases. Only further experience with this test will indicate its usefulness in detecting early diabetes as compared with that of the standard oral glucose tolerance test.

SCREENING PROCEDURES FOR THE DETECTION OF DIABETES MELLITUS

URINALYSIS

Routine urinalysis will lead to the detection of many unsuspected cases of diabetes mellitus. The finding of glucosuria is the most common, though not the most sensitive, indication that leads to a diagnosis of diabetes in routine practice. However, even if the urine specimen is obtained two hours after a high carbohydrate meal, glucosuria may be lacking in mild cases of diabetes. A postprandial blood sugar determination is preferable for routine screening for diabetes mellitus.

SINGLE BLOOD SUGAR DETERMINATION

Since the level of fasting blood sugar is in the normal range in many mild diabetics, this determination is an ineffective method of screening for diabetes mellitus. The two-hour postprandial blood sugar, after ingestion of a high carbohydrate breakfast (or 100 gm. of glucose) is a practical and much more sensitive method that leads to a diagnosis of previously unsuspected diabetes (see page 405). For convenience the

blood sugar may also be obtained ½ or 1 hour after a high carbohydrate meal. In this case, if the blood sugar is above 150 mg./100 ml. a glucose tolerance test is recommended.

GLUCOSE TOLERANCE TEST

Since performance of the glucose tolerance test is time-consuming, laborious, and expensive it is not a practical procedure for routine screening in office or hospital, or for mass screening. However, since the test is more sensitive than the two-hour postprandial blood sugar it should be used as a routine procedure in certain groups of individuals in whom experience has shown a high incidence of unsuspected diabetes mellitus. These groups have been discussed under "Indications for Glucose Tolerance Tests." For instance, among 400 apparently healthy close relatives of diabetic patients a 19 per cent incidence of previously unsuspected diabetes and a 3.5 per cent incidence of probable diabetes has been found. Efforts directed at detection of new diabetics are much more productive and rewarding when applied to relatives of diabetics than to the population at large.

POSSIBLE PREDICTION OF DIABETES MELLITUS

CORTISONE-GLUCOSE TOLERANCE TEST

Experience has shown that many nondiabetic relatives of diabetic patients will become diabetic themselves in the future. It is most desirable to be able to recognize this *potentiality* for the development of this disease. A cortisone-modified glucose tolerance has been devised that may accomplish this. After performance of an initial standard oral glucose tolerance test, 100 mg. (or 125 mg. if body weight above 160 pounds) of cortisone acetate are administered orally in two evenly divided doses 8½ and 2 hours preceding a second glucose tolerance test. A "negative" cortisone-glucose tolerance test is defined as one in which the two-hour value falls below 140 mg./100 ml. A "positive" cortisone-glucose tolerance test is one in which the two-hour value is at or above 140 mg./100 ml. Three of 104 control subjects without a family history of diabetes or large babies gave a positive response to the test. Among 265 nondiabetic relatives of diabetic patients 25 per cent gave a positive response (Fig. 30-2). Approximately 85 per cent of (1) patients with "probable diabetes" or (2) obese diabetic patients, whose glucose tolerance test had returned to normal after weight reduction, gave a positive response to the test. Only prolonged follow-up over many years will determine the value and reliability of the cortisone-glucose tolerance test in predicting future diabetes. At present, with a follow-up of

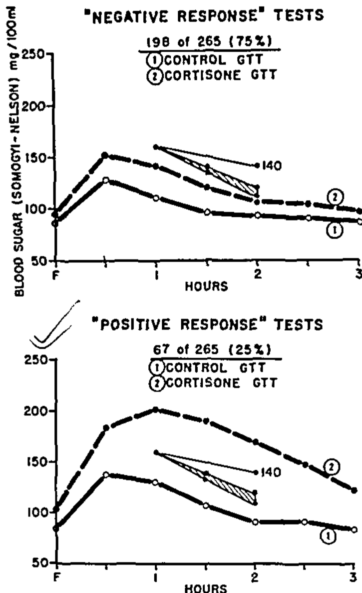


Fig. 30-2. Cortisone-glucose tolerance tests in nondiabetic relatives of diabetic patients. Three of 104 subjects without a family history of diabetes gave "positive responses" to the test.

1 to 6 years, 1 of 59 relatives who gave an initial negative response to the test has developed diabetes. On the other hand, of 40 relatives who initially gave positive responses to the test, 10 have developed diabetes and another 4 have glucose tolerance curves indicating probable diabetes.

An intravenous cortisone-glucose tolerance test using calculation of the "increment index" has been reported recently.

PREDNISONE-INDUCED GLUCOSURIA

Another possible approach to the evaluation of pancreatic insulin reserve by means of a standard, more prolonged glucocorticoid stress, has been suggested (Thorn, Renold, and Winegrad). In this test 24-hour glucose excretion is estimated by means of a specific glucose oxidase method. Prednisone is administered in a dosage of 50 mg. per day (25 mg. before breakfast and 25 mg. before lunch) for 3 or 4 days. In normal subjects without a family history of diabetes, glucosuria on the first day of steroid administration rarely exceeds 5 gm. The glucosuria decreases on subsequent days and returns to normal by the third day. In frankly diabetic patients the administration of prednisone results in a much greater glucosuria during the period of steroid administration. This test may unmask previously undetected diabetes and potential diabetes. However, the distinction between the mildly diabetic and the potentially diabetic patient may be impossible to make by estimation of glucosuria.

ANCILLARY TESTS FOR EVALUATION OF ABNORMALITIES OF CARBOHYDRATE METABOLISM

CHANGES IN LEVELS OF SERUM ELECTROLYTES AND INTERMEDIARIES OF CARBOHYDRATE METABOLISM

Attempts have been made to use changes in levels of serum phosphorus and potassium and blood pyruvate and lactate that accompany the disposal of glucose as an aid in the differentiation of mild diabetes mellitus from other diseases associated with abnormalities of carbohydrate tolerance, such as hepatic dysfunction.

Serum Phosphate and Potassium

Peripheral glucose utilization has been found to be associated with decreases in serum levels of inorganic phosphate and potassium following administration of glucose to normal subjects. In severely diabetic patients such changes have been reported to be less pronounced. However, the values for maximum fall in serum inorganic phosphate and potassium in mild diabetics are similar to those found in normal subjects and do not aid in the diagnosis of mild diabetes mellitus.

Blood Pyruvate and Lactate

In patients with severe diabetes, from whom insulin has been withheld, administration of glucose causes no rise or a delayed and smaller rise in blood levels of pyruvate and lactate as compared with normal subjects. In mildly diabetic patients increases in blood pyruvate and

lactate levels are not sufficiently distinct from responses seen in normals to be of diagnostic value.

However, in individuals with loss of glucose tolerance due to Cushing's syndrome or administration of adrenal corticosteroids there are marked increases in levels of pyruvate and lactate in the fasting state and further striking and rapid increases following ingestion of glucose.

INSULIN TOLERANCE TEST, INSULIN-GLUCOSE TOLERANCE TEST, GLUCAGON-RESPONSE AND EPINEPHRINE-RESPONSE TESTS

The following tests may contribute to the study of carbohydrate metabolism but are not helpful in the diagnosis of diabetes mellitus.

Insulin Tolerance Test

The insulin tolerance test has been used primarily to investigate (1) sensitivity or resistance to insulin and (2) responsiveness to insulin-induced hypoglycemia in patients with endocrinologic diseases associated with disturbances of carbohydrate metabolism. The test is performed after the subject has ingested a high carbohydrate diet for 3 days. After an overnight fast of 10 hours a fasting blood sugar is obtained and insulin (glucagon-free) is given intravenously in a dosage of 0.1 unit per kilogram of ideal body weight. In patients with suspected adrenal or pituitary insufficiency ½ to ⅓ of the calculated dose has been recommended to avoid dangerous hypoglycemia. Blood samples are obtained at 20, 30, 45, 60, 90, and 120 minutes after injection of insulin. Normally the level of blood sugar falls to about 50 per cent of the fasting level between 20 and 30 minutes and has returned to normal levels between 90 and 120 minutes after injection of insulin (Fig. 30-3).

Resistance to insulin is observed in some patients with acromegaly. A diabetic glucose tolerance test and a decreased hypoglycemic response to insulin have been used as indexes of excessive growth hormone secretion in such patients. Insulin sensitivity returns to normal after successful therapy of acromegaly (Fig. 30-3). Insulin resistance is also seen in Cushing's syndrome.

Patients with diabetes mellitus have been classified into insulin-sensitive and insulin-insensitive groups, depending upon their response to the blood sugar-lowering effect of insulin. The glucose-insulin tolerance test (see below) has also been used to make this distinction. In general, insulin sensitivity is observed in juvenile or growth-onset types of diabetic patient while insulin resistance is found in the middle-aged, obese or maturity-onset type of diabetic. However, there is considerable overlap between the two groups in their responses to these tests.

Patients with frank adrenal or pituitary insufficiency may have greater

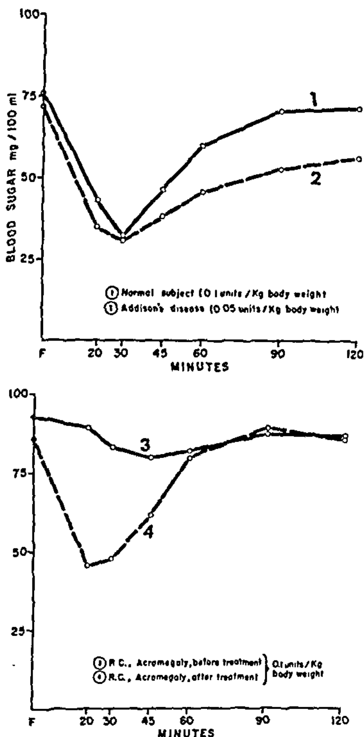


Fig. 30-3. Insulin tolerance tests. Curve 1, normal test in control subject, Curve 2, Addisonian patient with relative hypoglycemia-unresponsiveness following injection of half the usual dose of insulin/kg. body weight; Curve 3, acromegalic, before therapy, demonstrating insulin resistance, Curve 4, same acromegalic, after therapy, with return of normal insulin sensitivity.

than normal decreases in blood sugar and, more characteristically, a delayed return or lack of return of the blood sugar to normal (hypoglycemia-unresponsiveness) (Fig. 30-3).

Patients with primary myxedema may have a delayed and diminished decrease in blood sugar and a delayed return to initial blood sugar levels.

Glucose-Insulin Tolerance Test

In patients with diabetes the glucose-insulin tolerance test has also been used to determine whether the hyperglycemia is due to lack of insulin or to resistance to insulin. The test is performed like the regular

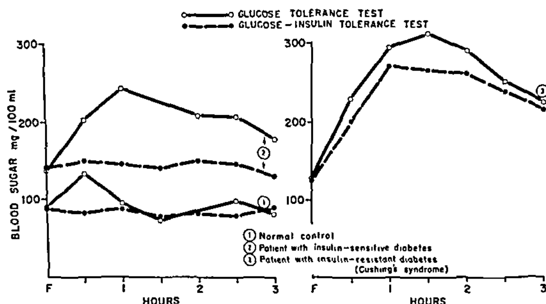
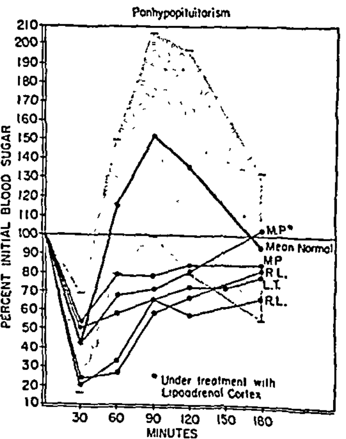


FIG. 30-4. Glucose-insulin tolerance tests and glucose tolerance tests: (1) normal control subject, (2) patient with insulin-sensitive type of diabetes, and (3) patient with insulin-resistant type of diabetes owing to Cushing's syndrome.

insulin tolerance test. In addition, 1 gm. of glucose per kilogram of body weight is given orally at the time of the insulin injection. In patients with resulting flat blood sugar curves it is assumed that the hyperglycemic response to glucose alone was due to insulin lack. Patients in whom the test shows little change from the hyperglycemic curve resulting from glucose administration alone are thought to have insulin resistance (Fig 30-4). This response may be seen in patients with Cushing's syndrome and in some patients with acromegaly.

The glucose-insulin tolerance test has been modified by Engel and



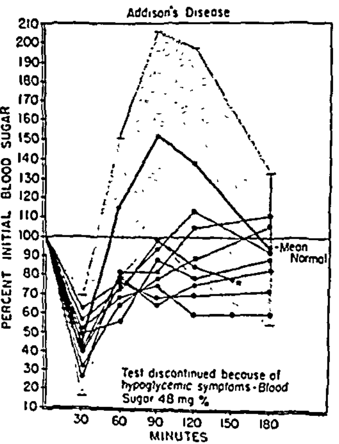


FIG. 30-5. Insulin-glucose tolerance curves in patients with panhypopituitarism and Addison's disease. The shaded area represents twice the standard *deviations from the* mean normal curve. (From Engel and Scott.)

Scott as a test for the detection of hypoglycemia-unresponsiveness in patients with possible pituitary or adrenal insufficiency. The insulin tolerance test is performed using 0.1 units of insulin per kilogram of body weight. Thirty minutes later, or earlier if significant symptoms of hypoglycemia appear, 0.8 gm. of glucose per kilogram is given orally. The procedure carries less risk of dangerous hypoglycemia than the conventional insulin tolerance test and magnifies the difference between normal and hypoglycemia-unresponsive patients. In patients with adrenal or pituitary insufficiency the blood sugar level does not rise as rapidly from hypoglycemic levels when glucose is given (Fig. 30-5). More direct tests to evaluate adrenal and pituitary function are available.

Glucagon Test

Administration of glucagon results in a rise in blood sugar level by stimulation of hepatic glycogenolysis. The degree of the hyperglycemic response to glucagon can be used as a measure of the adequacy of

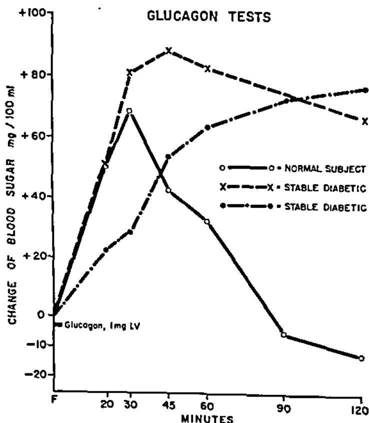


Fig. 30-6. Glucagon tests. Responses in a normal subject and in 2 patients with stable diabetes.

hepatic glycogen stores. One milligram of glucagon is administered intravenously over 3 to 10 minutes and blood samples are obtained at zero, 20, 30, 45, 60, 90, and 120 minutes. In normal subjects the level of blood sugar rises from 30 to 90 mg./100 ml. in 30 minutes and returns to control levels 90 minutes after the injection (Fig. 30-6). In patients with cirrhosis or with von Gierke's disease the response is greatly diminished or absent owing to defective glycogenolysis. Glucagon produces a more prolonged and sometimes a greater rise in blood sugar in diabetic than in normal subjects (Fig. 30-6). The literature contains conflicting results as to differences in the hyperglycemic response in stable as compared to unstable diabetics.

Epinephrine Test

Administration of epinephrine produces an elevation of blood sugar. This results primarily from hepatic glycogenolysis, but contributing factors are decreases in peripheral utilization of glucose and a decrease in muscle glycogen. As with the glucagon test the hyperglycemic response to administration of epinephrine has been utilized as a measure of hepatic glycogen storage.

NONDIABETIC MELITURIA

Melituria is the term applied to the presence of an abnormal amount of sugar in the urine. In addition to glucose, sugars such as fructose, lactose, galactose, maltose, pentoses, mannoheptulose, and sucrose may be found in urine. All the above sugars except the last one may give a positive reaction with a copper reduction method. Since the diagnosis of diabetes should never be made without blood sugar examinations (fasting blood sugar or carbohydrate tolerance test) a mistaken diagnosis of diabetes mellitus should never occur because of the appearance of glucose or other reducing substances in the urine of a nondiabetic subject.

GLUCOSURIA

Excretion of glucose in urine depends on (1) the concentration of glucose in the afferent glomerular arteriole (arterial blood), (2) the rate of glomerular filtration, and (3) the rate of renal tubular reabsorption of filtered glucose. Glucosuria occurs when glomerular filtration of glucose exceeds the maximal glucose reabsorptive capacity of the renal tubules. The blood sugar level at which glucose first appears in the urine is designated as the "renal threshold" for glucose for clini-

cal purposes. Normally glucosuria does not occur unless the venous blood sugar rises above 160 mg./100 ml. The "renal threshold" for glucose may be altered by changes in either glomerular filtration or tubular reabsorption of glucose. Usually a "lowered renal threshold for glucose" is due to decreased tubular reabsorption. An elevated renal threshold is most commonly due to decreased glomerular filtration.

Renal (Nondiabetic) Glucosuria

Renal or nondiabetic glucosuria is said to exist when the urine contains glucose in the presence of normal blood sugar levels and normal glucose tolerance. The defect may exist to a variable degree, some patients having glucosuria only after meals, while others have glucosuria even in the postabsorptive state. Renal glucosuria has been shown to be due to decreased tubular reabsorption of glucose. It is usually first recognized in healthy young people with otherwise normal renal function. The condition may be familial. The majority of authors have stated that the development of diabetes in individuals with nondiabetic or renal glucosuria is no more frequent than in the general population. However, in patients with nondiabetic glucosuria in whom follow-up glucose tolerance tests were performed up to 30 years later, unsuspected diabetes was found in 63 per cent of the cases. Approximately one third of the group had initially exhibited fasting glucosuria. This indicates that nondiabetic or renal glucosuria is not always a benign condition, that it is indicative of potential diabetes and that such patients should be retested periodically for the presence of diabetes mellitus. Table 30-7 shows examples of the transition from nondiabetic glucosuria to diabetes mellitus.

Renal glucosuria also occurs in a considerable proportion (10 to 15 per cent) of nondiabetic pregnant women. The glucosuria in these cases may be due either to increased glomerular filtration or to decreased tubular reabsorptive capacity for glucose or a combination of both factors. It must be stressed again that the finding of glucosuria in a pregnant woman should not be assumed to be renal glucosuria unless diabetes mellitus has been ruled out by a glucose tolerance test.

A special type of renal glucosuria is found in patients with the DeToni-Fanconi syndrome. These patients have not only lowered renal tubular reabsorptive capacity for glucose but also for amino acids and for phosphate. Hypophosphatemia, rickets or osteomalacia, and impaired growth are common. Ketonuria and a deficiency in ammonia production may also occur.

Nondiabetic glucosuria due to impaired reabsorption of glucose may

hepatic glycogen stores. One milligram of glucagon is administered intravenously over 3 to 10 minutes and blood samples are obtained at zero, 20, 30, 45, 60, 90, and 120 minutes. In normal subjects the level of blood sugar rises from 30 to 90 mg./100 ml. in 30 minutes and returns to control levels 90 minutes after the injection (Fig. 30-6). In patients with cirrhosis or with von Gierke's disease the response is greatly diminished or absent owing to defective glycogenolysis. Glucagon produces a more prolonged and sometimes a greater rise in blood sugar in diabetic than in normal subjects (Fig. 30-6). The literature contains conflicting results as to differences in the hyperglycemic response in stable as compared to unstable diabetics.

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cal purposes. Normally glucosuria does not occur unless the venous blood sugar rises above 160 mg./100 ml. The "renal threshold" for glucose may be altered by changes in either glomerular filtration or tubular reabsorption of glucose. Usually a "lowered renal threshold for glucose" is due to decreased tubular reabsorption. An elevated renal threshold is most commonly due to decreased glomerular filtration.

Renal (Nondiabetic) Glucosuria

Renal or nondiabetic glucosuria is said to exist when the urine contains glucose in the presence of normal blood sugar levels and normal glucose tolerance. The defect may exist to a variable degree, some patients having glucosuria only after meals, while others have glucosuria even in the postabsorptive state. Renal glucosuria has been shown to be due to decreased tubular reabsorption of glucose. It is usually first recognized in healthy young people with otherwise normal renal function. The condition may be familial. The majority of authors have stated that the development of diabetes in individuals with nondiabetic or renal glucosuria is no more frequent than in the general population. However, in patients with nondiabetic glucosuria in whom follow-up glucose tolerance tests were performed up to 30 years later, unsuspected diabetes was found in 63 per cent of the cases. Approximately one third of the group had initially exhibited fasting glucosuria. This indicates that nondiabetic or renal glucosuria is not always a benign condition, that it is indicative of potential diabetes and that such patients should be retested periodically for the presence of diabetes mellitus. Table 30-7 shows examples of the transition from nondiabetic glucosuria to diabetes mellitus.

Renal glucosuria also occurs in a considerable proportion (10 to 15 per cent) of nondiabetic pregnant women. The glucosuria in these cases may be due either to increased glomerular filtration or to decreased tubular reabsorptive capacity for glucose or a combination of both factors. It must be stressed again that the finding of glucosuria in a pregnant woman should not be assumed to be renal glucosuria unless diabetes mellitus has been ruled out by a glucose tolerance test.

A special type of renal glucosuria is found in patients with the DeToni-Fanconi syndrome. These patients have not only lowered renal tubular reabsorptive capacity for glucose but also for amino acids and for phosphate. Hypophosphatemia, rickets or osteomalacia, and impaired growth are common. Ketonuria and a deficiency in ammonia production may also occur.

Nondiabetic glucosuria due to impaired reabsorption of glucose may

TABLE 30-7. TRANSITION FROM NONDIABETIC GLYCOSURIA TO DIABETES MELLITUS

				Glucose tolerance test				
Subject and height	*Year*	*Age*	*Weight*	*Fasting*	*1 hr.*	*2 hr.*	*3 hr.*	*4 hr.*
R. K., female, 5'3"	1938	39	128	0 82	++ 112	+++ 95	+ 85	0 64
	1941	42	128	0 101	+++ 153	++++ 130	++++ 92	+++ 93
	1942	43	127	++++ 162	++++ 284	++++ 318	++++ 260	++++ 148
A. B., female, 5'4"	1936	22	114	0 89	+++ 135	++ 104	0 64	
	1948	34	115	++++ 260 (Polyuria, polydipsea, weight loss)				

occur in nephrosis with extensive degenerative changes in the renal tubules.

The normoglycemic glucosuria occurring during administration of large amounts of adrenal glucocorticoids to normal subjects has been shown to be primarily due to increased glomerular filtration.

Alimentary Glucosuria

Glucosuria is believed to occur in some individuals because of an increased rate of intestinal absorption of glucose. The resulting transient hyperglycemia exceeds the normal renal threshold, but the blood sugar returns to normal levels within two hours. There is no glucosuria at fasting blood sugar levels. Unless there has been gastric surgery, individuals with abnormally high peak blood sugar levels should be followed carefully by repeated glucose tolerance tests since this condition may be an early manifestation of diabetes mellitus (Table 30-4).

Glucosuria with Loss of Carbohydrate Tolerance

In addition to diabetes mellitus, glucosuria may occur in all conditions that cause decreased glucose tolerance as previously discussed.

FRUCTOSURIA

Essential fructosuria is a rare inborn error of metabolism characterized by the appearance of fructose in the urine in the absence of ingestion of large quantities of this sugar.

Fructose may also appear in the urine after ingestion of large amounts of this sugar in patients with liver disease and in a small

number of normal subjects (alimentary fructosuria). It also appears in patients with severe diabetes mellitus in association with glucosuria.

GALACTOSURIA

Congenital galactosemia is an inborn error of metabolism characterized by an inability to metabolize galactose. This leads to high blood levels and increased urinary excretion of this sugar after ingestion of milk. A specific enzymatic defect has been identified in liver and erythrocytes. Babies with galactosemia develop liver damage, mental retardation, and cataracts, probably secondary to accumulation of galactose-1-phosphate in the tissues. Evidence of abnormal carbohydrate metabolism and organ damage disappear when affected subjects are fed a milk-free diet early in life. In mild cases, galactosuria is not present and a diagnosis can be established by a galactose tolerance test. An enzymatic test performed on cord or venous blood has been described by which a diagnosis of congenital galactosuria can be made within 2 to 3 days of birth.

LACTOSURIA

Lactose appears in the urine of women during the period of lactation or immediately preceding it. This is a physiologic phenomenon.

PENTOSURIA

Essential pentosuria is a rare congenital defect in metabolism. Pentosuria may also occur in normal subjects after ingestion of large quantities of fruits with a high pentose content such as *plums, prunes,* grapes, cherries, and berries (alimentary pentosuria).

MANNOHEPTULOSURIA

Mannoheptulose may appear in the urine after ingestion of large amounts of avocado.

TESTS FOR KETONE BODIES IN URINE AND PLASMA

The so-called ketone bodies (acetone, acetoacetic and beta-hydroxybutyric acid) are present in traces in urine and plasma of normal individuals. These amounts are too small to be detected by the usual qualitative methods. When hepatic ketogenesis is increased and exceeds the capacity of the tissues to utilize ketone bodies, ketonuria becomes detectable. If hepatic ketogenesis exceeds both utilization and renal clearance, ketone bodies accumulate in the body fluids and become detectable in plasma.

Tests for Diacetic Acid and Acetone

ROTHERA TEST. To 5 ml. of urine add 1 gm. of ammonium sulfate or 1 ml. of a saturated solution. Add 3 drops of a freshly prepared 10 per cent solution of sodium nitroprusside. Mix and overlay with strong ammonia water. A purple ring indicates the presence of diacetic acid and acetone. The test is graded 1+ to 4+, depending upon the width of the ring, the intensity of the color, and the speed of its appearance.

ACETEST OR DENCO TEST. These are simplified adaptations of the nitroprusside test. Acetest Reagent tablets contain sodium nitroprusside, amino acetic acid, and disodium phosphate and lactose. A reagent tablet is placed on a piece of clean white paper and 1 drop of urine is placed on the tablet. The resultant color is read after 30 seconds. If the test is negative the tablet color will remain white or turn cream color from wetting. If the urine contains significant amounts of acetone the color of the tablet will change from a purple tint (1+) to lavender (2+) and then to a moderate purple (3+) or deep purple (4+), depending upon the amount present. The color may also be compared with the color chart and recorded as trace, moderate, and strongly positive.

Duncan has popularized a simple test for the detection of excessive ketone bodies in plasma or serum as an aid in evaluating the severity of ketosis and in gauging the insulin requirements during treatment of diabetic acidosis. Ketonemia does not become detectable until ketonuria has become 3+ to 4+. Conversely, a decrease in ketone content of plasma becomes detectable before any apparent reduction in ketonuria occurs. Ketonemia is gauged by placing a drop of undiluted plasma and plasma in various dilutions (50 per cent, 25 per cent, 12.5 per cent) on a portion of a crushed Acetest tablet. The color formed is read after 30 seconds. Ketonemia can be similarly evaluated by the Rothera-Wishart nitroprusside test.

KETOSTIX. A simple test for ketonuria or ketonemia by the use of a paper reagent strip impregnated with the same active ingredients as contained in the Acetest reagent tablets is available. The test end of the paper strip is dipped in urine or plasma and the color developed is compared with the color chart at 1 minute. Readings are similar to those used with the Acetest tablets.

Test for Diacetic Acid in Urine

GERHARDT TEST. To 5 ml. of urine add 10 per cent aqueous solution of ferric chloride drop by drop until the phosphates are precipitated, then add more. If diacetic acid is present the urine turns to a Burgundy-red

color. The test is graded according to the intensity of the color. False positive reactions may be given after ingestion of salicylates. Diacetic acid by this test will only become detectable after the nitroprusside test in urine has become 3+ or 4+ and thus it indicates a more severe grade of ketonuria. This test is used little today since the plasma ketone test is a better indication of the severity of ketosis.

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Chapter 31

ASSAYS OF INSULIN IN THE PLASMA

John Vallance-Owen

A reliable and reasonably accurate method for the estimation of the insulin concentration in blood plasma should prove useful for the elucidation of many problems in metabolism, particularly those relating to diabetes mellitus.

However, it should be immediately appreciated that the technical difficulties involved in this measurement are considerable. Not only is a very sensitive method required to measure the minute quantities of insulin present in plasma but any such method must be quantitative over the range of insulin concentration encountered. Moreover, it is probable that there are several substances present in plasma that modify the activity of the contained insulin; and a quantitative separation of the insulin from all possible interfering substances is difficult. Nevertheless, methods are available for measuring the effective insulin concentration, insulin activity, or free insulin in the plasma.

METHODS FOR INSULIN ASSAY

In Vivo Method

This method depends on the hypoglycaemic effect of insulin. The test animals used have been prepared in different ways to increase their

sensitivity. Thus adreno-demedullated, alloxan-diabetic, hypophysectomised rats (ADH rats), alloxan-diabetic, hypophysectomised, adrenalectomised rats (ADHA rats) and hypophysectomised alloxan-diabetic rats and mice (HAD rats and mice) have been used.

Apart from the technical difficulties involved in the preparation and maintenance of all these types of animal, which are considerable, their standardisation prior to the assay itself is extremely difficult.

The ADH rats are injected intravenously, under light nembutal anaesthesia, with insulin or with the material to be tested for insulin activity. The subsequent change in the blood sugar is measured over the next 30 minutes. These animals are given a glucose meal 20 minutes before the injection to prevent a possible fatal hypoglycaemia; this tends to produce a rising and variable base line blood sugar. This feature is unsatisfactory.

In order to get over the problem of anaesthesia and this rising base line, with HAD rats and mice the material under test is injected intraperitoneally 90 minutes after giving 300 mg. dextrose by stomach tube. The drop of blood sugar is then measured over the next hour.

Linear transformations of the dose-response relationship have been reported for each type of animal. However, these preparations have shown considerable differences in sensitivity and there has been a great deal of variation from animal to animal. These animals have, therefore, not been used extensively for the measurement of plasma-insulin activity.

The ADHA rat procedure has been used for this purpose. Insulin or whole plasma is injected subcutaneously and the effect measured over one hour, the test being performed when the blood sugar is constant. These rats are subject to mock assays with subcutaneous injections of saline to show that under these conditions the blood sugar is not altered. There was found to be a linear relationship between the log of the dose and the fall in blood sugar. However, standard doses of insulin were not included in the assay for plasma insulin activity and the method, as originally described, has not been confirmed. On the contrary, several investigators have reported an extremely high mortality during the preparative procedure, and found that even those animals which did survive maintained a blood sugar too unstable to permit their use for insulin assay.

In view of the unsatisfactory nature of the in vivo procedures, a relatively simple and inexpensive in vitro technique has been extensively studied in the last few years.

In Vitro Methods

RAT DIAPHRAGM TECHNIQUE. The most popular in vitro method involves the use of the isolated rat diaphragm. The method depends on the fact that small amounts of insulin increase the utilisation of glucose by the isolated rat diaphragm, and that a quantitative relationship exists between the concentration of insulin in the incubation medium, and its effects on the glucose metabolism of the diaphragm. By rigorous control of the experimental conditions this method becomes extremely sensitive, detecting as little as 10^{-5} units/ml. insulin, and thus can readily measure the small quantities of free insulin in the plasma. This contention has been adequately and repeatedly confirmed.

The techniques used by the various investigators in this field are essentially similar, though varying somewhat in practical detail. Briefly, the uptake of glucose from undiluted or diluted plasma is compared with the uptake from buffer solution of similar ionic composition to plasma with and without added insulin. Satisfactory linear transformations of the dose-response curve have been reported and confirmed. The slopes of such dose-response regression lines ordinarily do not differ significantly though varying in position from day to day. Consequently, appropriate standards of insulin must be incorporated in each assay in order to fix the position of this dose-response line. The glucose uptake from the plasma samples can then be compared directly with this line and the insulin activity read off.

It is likely that one of the most important differences in procedure has been in the handling of the plasma, namely its conditions of collection and its modification prior to use as an incubation medium. This aspect will be considered more fully when dealing with the specificity of the assay.

This method is simple and reproducible. Its index of precision, although not high, is satisfactory, varying between 0.17 and 0.4, and there has been, in the author's hands, only a 20 per cent error in the estimation of unknown samples of insulin solution.

Other Possible Methods

The glucose uptake of the epididymal fat pad, chick fibroblasts, or the perfused rat heart also respond in the same way as the rat diaphragm to small doses of insulin. Thus such systems could be used for plasma insulin assay provided the conditions can be adequately controlled, though they are unlikely to be more sensitive than the isolated diaphragm or to possess any particular advantage.

The gas output of lactating mammary glands from the rat is increased by insulin, but the sensitivity of this system is apparently not high.

Immunologic Techniques

There appears to be a great future for techniques based on antigen-antibody reactions. Although insulin is ordinarily a poor antigen, potent antiserum can be prepared against it in guinea pigs by injection of large doses subcutaneously in oily suspension with Freund's adjuvant. Also, anti-insulin serum can be made in rabbits by repeated small injections of alum-precipitated insulin.

The antiserum formed in these ways can be used to estimate insulin activity by employing the optimum proportions in which insulin and antibody combine together. The most satisfactory technique for demonstrating this combination is to use an indicator of any excess insulin, and this is provided by tannic acid coated red cells that lyse in the presence of insulin. Another method, rather less satisfactory, has been the protective effect of the antiserum on animals.

Although there is some antigenic crossover from species to species, this is by no means absolute. Hence it is not permissible to use antiserum prepared against the insulin of one species to measure that of another. This method should measure the absolute amount of insulin whereas the other types of assay measure the effective insulin concentration or the insulin activity in plasma.

SPECIFICITY OF METHODS OF ASSAY

*It has been clearly shown that plasma contains factors other than in*sulin that can affect the assay procedures (See Chaps. 19, 20). Thus it is again emphasized that the results obtained using whole plasma with the in vivo methods and the rat diaphragm procedure measure the *effective* insulin concentration or insulin activity of the plasma, as it has been called. This is the sum of the biologic effect of insulin and its synergists, if any, on the one hand and its antagonists on the other. However, it is now apparent, from more recent experiments with the rat diaphragm, that dilution of whole normal plasma neutralises—in part at any rate—the effect of some insulin antagonist in undiluted plasma, or alternatively releases insulin from a bound inactive form. Thus if the plasma is diluted 1:4 before assay, the same glucose uptake is achieved as when the plasma is studied undiluted, but further dilution causes the expected fall in uptake. Therefore investigators using

undiluted plasma with the rat diaphragm technique have always obtained lower values for insulin activity than those using diluted plasma. This point is stressed as it has given rise to confusion not only over normal values, but also in the interpretation of results relating to the plasma insulin activity in diabetes mellitus.

The evidence which suggests that insulin activity is indeed being estimated in plasma by the rat diaphragm technique can be briefly summarised as follows:

1. After pancreatectomy in cats and dogs, there is no measurable plasma insulin activity.

2. The increased glucose uptake from standard insulin solutions and from plasma can be inhibited by cysteine and glutathione; insulin loses its activity in the presence of such SH compounds.

3. When regular insulin, 0.1 units/kg. body weight, is injected intravenously into normal fasting subjects, a considerably greater glucose uptake, and hence insulin effect, is found from plasma obtained 20 minutes after the injection than from plasma removed in the fasting state before the injection was given.

4. Patients with islet-cell tumours have excessively high levels of plasma-insulin activity, both in the fasting state and one hour after glucose by mouth. These levels fall into the normal range after the tumour has been removed.

5. Antiserum prepared against beef insulin in a guinea pig will abolish the increased glucose uptake from standard solution of beef insulin and from the plasma of a cow.

It has been suggested that the insulin-like action of plasma on the rat diaphragm was due to a nonspecific effect of plasma albumin. However, it is now clear that the stimulating effect of such albumin fractions was due to the contamination of the preparation with insulin itself.

PLASMA INSULIN ACTIVITY IN NORMAL HUMAN SUBJECTS

The values that have been obtained by different authors are shown in Table 31-1 For simplicity, only those investigators who have gone on to study plasma insulin activity in diabetes have been included.

In spite of the over-all variation, those investigators using the ADHA technique and whole undiluted plasma in the rat diaphragm procedure have obtained reasonably comparable results: up to 100 μu./ml. for fasting samples (microunit $= 10^{-6}$ international units). After oral glucose there was usually a considerable increase above the fasting level, up to sevenfold in plasma insulin activity.

TABLE 31-1. INSULIN ACTIVITY IN PLASMA FROM NORMAL SUBJECTS

Method of assay	*Plasma insulin activity μu./ml.*	*Investigator*
ADHA rat*	100 (fasting) 340 (2 hrs. after oral glucose)	Bornstein (1950)
Rat diaphragm with whole undiluted plasma	30-80 (fasting)	Vallance-Owen and Hurlock (1954)
	100-800 (1 hr. after oral glucose)	
	55 (mean fasting) >100 (1 hr. after oral glucose)	Wright (1957)
Rat diaphragm with diluted plasma	60-600 (Conditions not stated)	Groen, *et al.* (1952)
	100-3000 (Conditions not stated)	Willebrands and Groen (1956)
	1000-20,000 (fasting or 2 hrs. after oral glucose)	Randle (1954)

* ADHA Alloxan-diabetic, hypophysectomised, adrenalectomised.

PLASMA INSULIN ACTIVITY IN DIABETES

Interest has centered mainly around the two broad clinical groups of diabetic patients. In the first, the patients, besides having hyperglycaemia and glycosuria, usually develop ketosis and rapidly lose weight unless given insulin treatment. The patients in the second group *do not require insulin and show no tendency to ketosis unless their* diabetes is complicated by infection. These patients are usually obese and often recover from the diabetic state when they lose weight on a low carbohydrate reducing diet.

The ADHA rat in vivo technique and the rat diaphragm procedure have been used for the study of plasma insulin activity in these patients.

When the ADHA rat method was used, the untreated patients who had markedly elevated fasting blood sugar levels were all prepared in the same way. They were given 50 gm. oral glucose and the blood was collected 2 hours later, in order to stimulate any possible insulin secretion in a comparable manner.

The mild obese group all had insulin activity in their plasma. The mean activity was 230 μu./ml., which was about 70 per cent below the mean value of 340 μu./ml. found in normal subjects treated in the same fashion.

On the other hand, those patients classed as severe who were losing weight and who subsequently needed insulin to prevent ketosis had no measurable plasma-insulin activity. When the patients in this group re-

ceived insulin treatment so that their blood sugar was physiologic at the time of the test, insulin activity was found in their plasma at a mean level of 230 μu./ml.

It was also noted that there was another clear difference between these groups: the ADHA rats used for testing the inactive diabetic plasma were rendered insensitive to insulin in subsequent control tests. This was not the case with the rats used for the plasma from the obese group that had insulin activity. There was no insulin activity in the plasma of two insulin-resistant diabetics whose blood sugar was elevated even 1 hour after an injection of 300 units of insulin.

Investigators using whole undiluted plasma with the rat diaphragm technique have recorded somewhat similar results:

Insulin activity was found in the plasma of all patients in the obese diabetic group. The range in the fasting state was from 50–300 μu./ml. with an over-all mean of 125 μu./ml., which is slightly above that found in normal fasting subjects. Increased insulin activity was usually found after glucose. The range now was from 250 to 600 μu./ml., with a mean of 430 μu./ml., which was not dissimilar to that found in normal subjects under the same conditions.

When insulin was added in vitro to the plasma from these diabetics, the activity of the added insulin was not diminished; i.e., there was no demonstrable antagonism, which is similar to the findings in normal subjects.

Turning now to the group of diabetics who require insulin treatment to prevent ketosis: if these patients were untreated or uncontrolled at the time of the test, no plasma insulin activity was found, even though they might have received a substantial injection of insulin 1 hour before the blood was withdrawn for testing. Moreover, when insulin was added in vitro to the plasma from these patients, the activity of the added insulin was inhibited and markedly diminished.

If, however, these patients were controlled with insulin, so that their blood sugar level was physiologic at the time of the test, plasma insulin activity was again found, essentially in the normal range, and insulin added in vitro was no longer inhibited. It must be stressed again that all the above observations were carried out using undiluted plasma for the assay. If inhibiting plasma with no insulin activity from uncontrolled insulin-requiring diabetics was diluted 1:4 prior to the assay, then insulin activity was found in this plasma and now there was no inhibition of any additional insulin. This observation explains the findings of investigators who have studied diabetic patients, using diluted plasma in the assay procedure. Such investigators have found no difference between the two diabetic groups or between them and

normal subjects with respect to plasma insulin activity, except in diabetic coma, when no insulin activity was found.

The results obtained in diabetic patients by the in vivo and in vitro techniques are summarized in Table 31-2.

TABLE 31-2. INSULIN ACTIVITY OF PLASMA FROM DIABETIC PATIENTS

Type of diabetic	*Method of assay*	*Plasma insulin activity μu./ml.*	*Investigator*
Mild obese	ADHA rat	230 (2 hrs. after glucose)	Bornstein and Lawrence (1951)
	Rat diaphragm with undiluted plasma	50–300 (fasting) 250–600 (1 hr. after glucose)	Vallance-Owen *et al.* (1955)
	Rat diaphragm with undiluted plasma	Normal levels (fasting and after glucose)	Wright (1957)
	Rat diaphragm with diluted plasma	Normal levels	Willebrands and Groen (1956)
Insulin-requiring			
Untreated	ADHA rat	None detected } 2 hrs. after glucose	Bornstein and Lawrence (1951)
	ADHA rat	230 } 2 hrs. after glucose	
Untreated or uncontrolled	Rat diaphragm with undiluted plasma	None detected	Vallance-Owen *et al.* (1955)
Well controlled	Rat diaphragm with undiluted plasma	Normal levels	
Untreated or treated	Rat diaphragm with diluted plasma	Normal levels	Groen *et al.* (1952) Willebrands and Groen (1956)

DISCUSSION OF THE RESULTS OBTAINED IN DIABETIC PATIENTS

Mild Obese Diabetics

Although near normal values for plasma insulin activity have been found in the mild obese group of diabetics, it would be incorrect to believe that the pancreas is therefore acting normally. The amount of insulin produced by the pancreas of a normal subject against the blood sugar level ordinarily maintained by these diabetics would probably be very much greater than is actually found in these patients. This point has not as yet been investigated by direct measurement of insulin activity. However, there is good indirect evidence that more insulin is

produced under such circumstances by normal subjects, derived from glucose assimilation studies following intravenous infusions of glucose. In other words, sufficient insulin can be produced by the pancreas of these mild diabetics for ordinary requirements, but there is less than the normal functional reserve. This is inadequate with obesity, when more insulin is required for normal metabolism—hence the amelioration of the diabetic state with weight reduction in many of these patients.

When insulin is added in vitro to the plasma from these patients there is no demonstrable antagonism. Thus the well-known resistance to insulin which these obese diabetics exhibit presumably resides in the tissues.

Insulin-Requiring Diabetics

When such patients are uncontrolled, there is no measurable plasma insulin activity and any insulin added to their plasma in vitro is inhibited.

This observation suggests that these diabetics require insulin to overcome some antagonist circulating in their plasma. In order to achieve control, sufficient insulin must be given to counteract this antagonism and yet leave sufficient active insulin to carry out its normal functions. It is entirely possible, at least initially, that there is some endogenous insulin production in these patients that is completely obscured by the antagonistic activity. *The insulin antagonism found in these uncontrolled* insulin-requiring diabetics may be an exaggeration of the situation in normal subjects, where the antagonism is completely masked by adequate insulin production by the pancreas. In animals, this antagonism can be revealed by pancreatectomy.

It has recently been shown that the antagonism to insulin found in the plasma of these patients resides in the albumin fraction of the plasma proteins. Similar antagonism has also been found in the plasma albumin fraction from normal subjects, but the fraction from normal subjects is less active in this respect. Radioactive studies have shown that insulin can be bound to albumin. On this basis it is possible to explain the difference between the antagonistic activity of the normal and diabetic albumin. The normal albumin will have some insulin bound to it whereas the diabetic albumin will be relatively free of insulin. Another explanation for the difference would be that the antagonism is due not to albumin per se but to some substance tightly bound to it, in larger amounts on diabetic albumin than on normal albumin. This possibility receives some support from the observation that the plasma albumin fraction from hypophysectomised patients is

devoid of antagonistic activity. However, the pituitary gland may not be solely responsible for the demonstrated antagonism to insulin in plasma, as can be seen from studies made on experimental diabetes in animals.

EXPERIMENTAL DIABETES IN ANIMALS

As with normal subjects, the plasma from normal fasting cats had insulin activity but no measurable antagonism to added insulin.

There was no insulin activity in the plasma from depancreatised cats, as might be expected, and such plasma also inhibited the activity of any insulin added in vitro; i.e., an experimental situation was obtained that was not dissimilar to that found with uncontrolled insulin-requiring diabetic patients.

TABLE 31-3. INSULIN ANTAGONISM OF PLASMA FROM DIABETIC PATIENTS AND CATS

Species and type of diabetic	*Antagonism of insulin added in vitro*	*Investigator*
Man		
Mild obese	No	Vallance-Owen *et al.* (1955)
Insulin-requiring		
Uncontrolled	Yes	
Controlled	No	
Cat		
Normal	No	Vallance-Owen and Lukens (1957)
Depancreatised	Yes	
Depan -Hypox.*	No	
Depan -Hypox. + F*	No	
Depan -Hypox. + G H *	No	
Depan -Adrex.*	No	
Depan -Adrex + E or F*	Yes	
Depan -Adrex. + G H *	No	

* F = Hydrocortisone acetate, E = Cortisone acetate, G H = Growth hormone; Hypox. = Hypophysectomised; Adrex = Adrenalectomised

This inhibition of insulin in the plasma from depancreatised cats could be abolished either by hypophysectomy or by bilateral adrenalectomy.

In either depancreatised-hypophysectomised (Houssay) or depancreatised-adrenalectomised (Long-Lukens) cats, there was again no plasma insulin activity but now the activity of insulin added to the plasma was fully recovered.

Cortisone or hydrocortisone, 10 mg. per day, injected subcutaneously

for 4 days into the Long-Lukens type of animal, restored the inhibitory properties of the plasma. The same dose of hydrocortisone injected into Houssay cats for 4 days and even longer, until severe ketosis was produced, did not restore the inhibitory activity of the plasma from these animals.

Growth hormone (3 mg./day for 4 days) injected subcutaneously into either Long-Lukens or Houssay cats failed to restore the inhibiting properties of the plasma. Table 31-3 summarises the results, which indicate that both the pituitary gland and the adrenal oxysteroids must be present for insulin antagonism to be found in the plasma of depancreatised cats. It is not known, as yet, whether the antagonism in these animals is carried in the same plasma protein fractions as that found in normal subjects and insulin-requiring diabetics.

In view of the apparent arrest of diabetic complications by hypophysectomy or adrenalectomy, the above observations have more than theoretical interest, for through further study of insulin antagonism we may learn something more, not only about the abnormal metabolic state of diabetes but also about the cause and subsequent prevention of the serious complications often associated with this disease.

GENERAL CONCLUSIONS

There are now available several highly sensitive methods for measuring insulin activity and antagonism in plasma. Although none of them is entirely satisfactory, they have yielded important results in relation to insulin activity in diabetes mellitus.

The mild obese group of diabetics, who do not require insulin treatment, have near normal levels of plasma insulin activity in the fasting state. There is a rise in activity after glucose by mouth as in normal subjects and also added insulin is satisfactorily recovered. Thus the well-known resistance to insulin exhibited by these patients does not reside in the plasma and presumably is present in the tissues.

Untreated or uncontrolled insulin-requiring diabetics have no measurable plasma insulin activity. Moreover, when insulin is added to the plasma from these patients, its activity is apparently inhibited. If the patients in this group are controlled, however, plasma insulin activity is again found, essentially in the normal range, and added insulin can now be recovered. In view of this antagonism, the amount of endogenous insulin produced by these patients is not known

The antagonism to insulin noted in these insulin-requiring diabetics, is probably an exaggeration of the situation in normal subjects where the antagonism is masked by adequate insulin production by the pan-

creas. In both situations the antagonism appears to reside in the albumin fraction of the plasma proteins and is related in some way to the pituitary gland.

This organ may not be wholly responsible, however. Experiments on depancreatised cats suggest that, in these animals at least, the presence of the adrenal oxysteroids is also necessary for the production of insulin antagonism in their plasma.

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Chapter 32

ASSAYS FOR INSULIN IN THE PANCREAS

Gerald A. Wrenshall

The classical work of Banting and Best established in 1921 that the pancreas contains an extractable *store of insulin. Since then* the magnitude of this deposit has been referred to as the *insulin content of the pancreas* and, where measurement involves extraction by a stated method, as the *amount of insulin extractable from the pancreas* (AIEP). The insulin extraction method used in obtaining the great majority of the results to be discussed in this chapter is the ethanol-HCl procedure of Scott and Fisher. This was followed by assay using a mouse-convulsion method. Methods employed for the assay of insulin are described in Chapter 31.

Reviews of the earlier and more recent publications on the pancreatic store of insulin have been made by Haist and by Best *et al.*, respectively. Publications dealing specifically with the AIEP in diabetic subjects are few, and are included in the reference list.

MANNER OF EXPRESSING RESULTS

The adult human pancreas is a tissue of highly variable composition and size. Balo found that the fat content of the pancreas in elderly subjects ranged between 2 and 74 per cent of the wet weight.

Owing to these circumstances the *concentration* of extractable in-

sulin, usually expressed as units of insulin per gram of pancreas, is not a satisfactory measure of the AIEP in man. In fact, an inverse correlation exists between the total weight of the nondiabetic human pancreas and the concentration of extractable insulin. The existence of such a relation is evidence that the *total* pancreatic reserve of extractable insulin is more characteristic of the subject than the concentration. This total AIEP has also been expressed as units of extractable insulin per unit of body weight, height, or surface area.

Biologic variation rather than inaccuracies in measurement is the principal source of the large variation (standard deviation ±40 per cent of the mean) that still remains in measurements of the AIEP in nondiabetic human subjects. Some of this variation is undoubtedly related to abnormal nutritional conditions causing changes in the concentration of extractable insulin in the β-cells. However, Ogilvie has found an equally large variation (standard deviation ±42 per cent of the mean) in the *volume* of islet tissue in nondiabetic subjects, so that variability in islet volume appears to be the major source of scatter in values for the AIEP of nondiabetic man.

INTERPRETATION OF AIEP MEASUREMENTS

Haist has reviewed evidence relating the effects of nutritional and endocrine factors to the AIEP of experimental animals. His concept of the AIEP as the contents of a metabolic compartment in which the insulin is being turned over is illustrated in Figure 32-1. At any point in time the AIEP must represent the accumulated difference between the rate of appearance of insulin in and the rate of its removal from the compartment.

The major if not the only way in which new insulin appears in the pancreas compartment is by synthesis. It can be shown that, even if it could occur, reaccumulation by the pancreas of all circulating insulin could not increase the average pancreatic store by as much as 7 per cent of that already there, and this is much smaller than the observed range of variation in values for the AIEP in adult human subjects. Insulin disappears from the pancreas by transfer into the blood for removal by other tissues (Chap. 4). The possibility that some insulin may be destroyed as such within the pancreas compartment has not yet been ruled out. An increase in the AIEP means, therefore, that the rate of insulin synthesis has been greater than the rate of insulin disappearance from the pancreas. Such an increase can occur by an increase in the concentration of insulin in the pancreas compartment, an increase in the volume of the compartment, or both.

The role of the β-cells of the islets of Langerhans in the synthesis and storage of insulin has been described elsewhere (Chaps. 4 and 25). Relating these cells to the storage of insulin as described above, an increase in the concentration of stored insulin in the pancreas compartment corresponds to an increase in concentration of insulin, in fixed or unfixed form, in the existing β-cells. An increase in the volume of the compartment corresponds to hypertrophy and/or hyperplasia of the β-cells.

Conversely, a decrease in the AIEP must mean that the rate of insulin synthesis has been less than the rate of insulin removal from the pancreas. This could occur in the β-cells as a decrease in the concentration

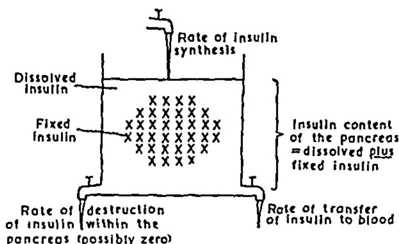


FIG. 32-1 Schematic representation of the endocrine pancreas relative to the production, storage, and removal of insulin. Factors determining the rates of insulin synthesis and removal are indicated as handles on the taps.

of stored insulin, or as atrophy and/or hypoplasia of the β-cells. It will be apparent that, in the absence of workable tracer methods, an observed change in the AIEP requires knowledge of the quantity and state of the β-cells as well as of the metabolic condition of the subject, if it is to be interpreted even qualitatively in terms of insulin production in and removal from the pancreas.

Using Wilson's modification of Gomori's aldehyde-fuchsin technique, a direct proportionality is found to exist between the amount of stainable granular substance in the cytoplasm of the β-cells and the AIEP, both amounts being expressed per unit weight of pancreas. The proportionality factor is different for diabetic and nondiabetic human subjects compared as two groups. This difference is such that a smaller

fraction of the AIEP appears as stainable granulation in the diabetic pancreas than in the nondiabetic pancreas.

Presumably insulin fixed in the β-cell granules passes through a non-fixed phase insensitive to the aldehyde-fuchsin staining technique before passing out of the β-cell cytoplasm. In these terms this shift might represent insulin passing into a soluble form under the action of some insulin-mobilizing agent, possibly glucose, which is in higher concentration in the plasma of the average diabetic than in the average nondiabetic subject.

NONDIABETIC SUBJECTS

The amount of insulin extractable from the whole pancreas increases continuously with age from early childhood, reaching the adult level between the twelfth and sixteenth years of life. In our experience the average concentration of extractable insulin falls from about 4 units per gram of pancreas at one year of age to 2.3 units per gram in adult subjects. However, accompanying this fall there is an increase in the average pancreas weight from about 9 Gm. at one year of age to 87 Gm. in adult subjects. Thus, on the average, the *total* amount of insulin extractable from the pancreas increases fivefold to sixfold from one year of age to its maximum value in the period of greatest growth. The only notable trend following the period of normal body growth is a slight decrease in the AIEP after 50 years of age.

The above changes in the AIEP during childhood and adolescence are accompanied by a corresponding increase in islet mass caused almost entirely by islet hyperplasia (Ogilvie). According to Hartroft, the abundance of β-cells, rather than the fairly uniform concentration of their cytoplasmic granular substance, parallels the concentration of extractable insulin in nondiabetic subjects. On the other hand, both factors are important in demonstrating the correspondence between concentration of granular substance in β-cell cytoplasm and concentration of extractable insulin within the diabetic pancreas.

DIABETIC SUBJECTS IN GENERAL

The AIEP at autopsy in all male and female diabetic human subjects of the present Toronto series is shown as vertical lines and empty circles in the pair of diagrams of Figure 32-2, as a function of the *age at diagnosis of diabetes mellitus* and the *duration of life between diagnosis and death*. Where less than 0.1 units of insulin per kilogram of body weight could be extracted from the pancreas (i.e., less than 3 per

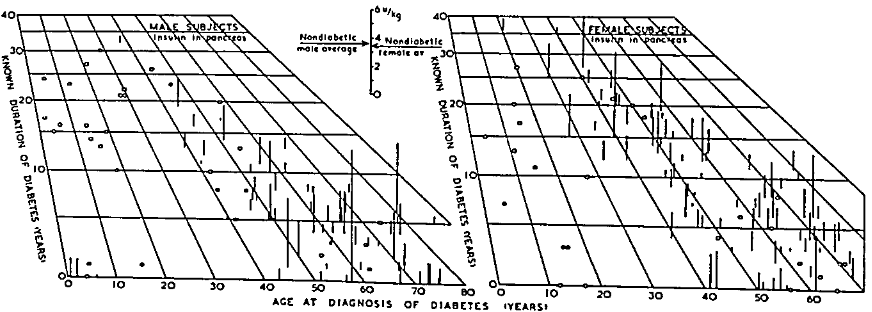


FIG. 32-2. The amount of insulin extractable from the diabetic human pancreas at autopsy (units per kilogram of body weight shown as vertical lines) plotted for each sex against age at diagnosis and duration of diabetes mellitus. Open circles represent very low values (less than 0.1 units/kg. of body weight).

cent of the average level found in the corresponding nondiabetic series), the value is plotted as an empty circle. Certain general characteristics of these 3-dimensional graphs become apparent on inspection or upon statistical analysis. Subjects who were diagnosed diabetic within the age range where growth normally occurs (approximately 0–20 years) have a gross lack of extractable insulin. Only in two *newly* diagnosed subjects of this growth-onset group were more than trace-amounts of insulin extractable from the pancreas.

A concurrent study of histologic sections taken from the pancreas of each of these subjects revealed that, in all cases where the subject had survived for more than a year following diagnosis of diabetes, β-cells were either very scarce or none at all was found. Where a few β-cells could be identified, they were almost devoid of stainable cytoplasmic granular substance. In two out of the six growth-onset diabetic subjects who died shortly after diagnosis, the abundance of β-cells was reported as normal but degranulation had occurred.

Consideration of the figures indicating the AIEP and of the histologic pictures in the same pancreas consistently leads to the conclusion that, *in growth-onset diabetes of more than a few months' duration, the AIEP is very small because of an almost complete lack of production of endogenous insulin.* In terms of the combined histologic and insulin assay findings, *none of the 13 growth-onset subjects of this series who survived beyond the period of normal growth recovered the ability to produce insulin.*

In the two newly diagnosed subjects with normal numbers of β-cells but with the AIEP reduced, it can only be said that the rate of secretion of insulin had exceeded the rate of its synthesis for a time prior to death, thus reducing the AIEP. This reduction could have come about either by a reduced ability of the pancreas to synthesize insulin, by an increased release of insulin by the pancreas, or by both processes acting together.

Different levels and patterns for the AIEP and β-cell histology are seen for subjects diagnosed diabetic after the normal period of skeletal growth is past (maturity-onset diabetes). In a few subjects of both sexes the AIEP is larger than the average value for nondiabetic adult subjects. In some others it is very low, as was the case for growth-onset diabetic subjects. *On the average* the AIEP in maturity-onset diabetics amounts to about 40 per cent of that in adult nondiabetic subjects (Fig. 32-2).

If the female maturity-onset subjects are considered *as a group,* there is no statistically significant change in the AIEP with either age at diagnosis or known duration of diabetes. When the male maturity-onset subjects are similarly considered, a general increase in the AIEP with

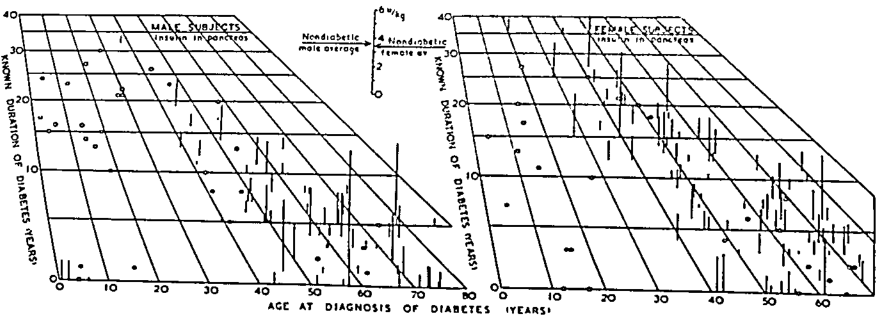


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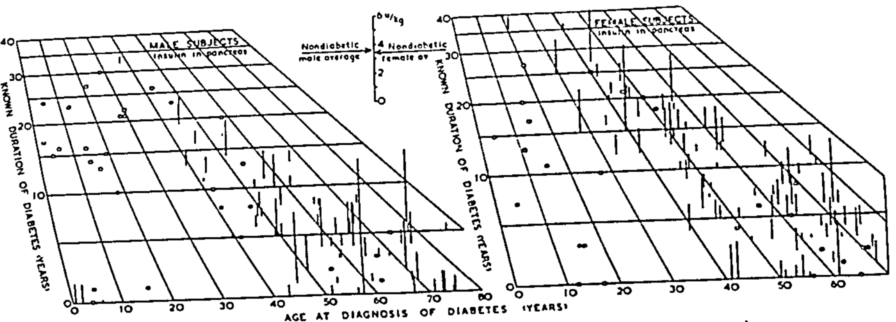


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Chapter 33

DIET AND GENERAL CARE

Alexander Marble

AIMS OF TREATMENT IN DIABETES

Expressed as ideals toward which to strive, the aims of treatment of diabetes are to abolish glycosuria; to keep the blood sugar as well as the blood cholesterol and lipids within normal bounds; to prevent ketoacidosis and coma; to maintain weight, strength, and health at a level as nearly normal as possible for the age and general status of the individual concerned; and to prevent late, degenerative complications of the vascular and nervous systems. With the means now available, it is rarely if ever possible to attain these ideals completely. However, for the physician to have them in mind as he sees each patient serves to orient and direct treatment along the best possible channels. The lower the goal the physician sets, the less likely he is to achieve satisfactory results.

In the attempt to reach the ends just outlined, the following are essential: (1) A carefully planned diet suited to the individual and thoroughly adequate in protein, vitamins, minerals, and calories. (2) Insulin of appropriate type and dosage in those patients with whom dietary restriction alone will not suffice for control. Experience in recent

to the β-cells will answer some of our questions. Improved methods for the assay of plasma insulin and for the estimation of the rate of blood flow from the islet cells would solve other aspects of the problem of the production and release of insulin.

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years suggests that oral hypoglycemic agents may be used instead of insulin in carefully selected patients. (3) Physical activity suited to the individual. (4) Education of the patient and his family.

The degree of control actually obtained will depend upon the quality of treatment provided, the degree of co-operation of the patient, and the character of the diabetes. *With many patients taking insulin,* hyperglycemia must be allowed at times during the day to avoid hypoglycemic reactions at others. Various compromises and deviations from the ideal must of necessity be made. However, with careful treatment begun soon after onset of the disease and with co-operation on the part of the patient, such *good results are usually obtained that the diabetic condition ceases to be a significant handicap and the individual is able* to take a fully satisfying place in the life and work of his community.

GENERAL PRINCIPLES OF DIET

Regardless of other forms of therapy, the basis of all treatment of diabetes is a carefully planned diet. The proper approach to its prescription, as with any special diet, is to regard it as a modification of a normal diet with due consideration of the dietary habits of the patient concerned. In addition to arranging for appropriate amounts of carbohydrate, protein, fat, and calories, the necessity for providing adequate amounts of vitamins and minerals must be kept prominently in mind

CARBOHYDRATE. *Prior to the introduction of insulin in 1922, diets* prescribed for diabetic patients rarely provided more than 100 gm. of carbohydrate a day and often, considerably less. Because of the ability, conferred by insulin, of utilizing much larger amounts of carbohydrate, there has been a considerable difference in opinion and practice among *physicians as regards the most desirable* level of carbohydrate intake. Some have allowed as much as 300 gm. or more of carbohydrate daily with concomitant lowering in the amount of fat. Some have even advocated a "free diet," allowing the patient to eat as he would if he did not have diabetes. However, most proponents of this program direct their patients to omit concentrated sweets and pastries.

Experience gained in the past 37 years has led to greater rather than less uniformity in the prescription of diets throughout the world. It is fair to state that *today most clinicians interested in diabetes prescribe* for their adult patients diets with a carbohydrate content ranging between 150 and 200 gm. daily, exclusive of the carbohydrate contributions of protein and fat. In the practice of the author and his colleagues, this range is followed in general with the average diet for adult patients *calling for* 150–180 gm. of carbohydrate daily. Patients on low-calorie

diets designed for weight reduction may receive as little as 120–130 gm. a day. Young adult patients engaged in strenuous labor as well as older and active diabetic children may be given as much as 225 gm. of carbohydrate a day.

The advantages of providing for moderately liberal amounts of carbohydrate(150–200 gm. a day) are: (1) The diet is more palatable and, therefore, more likely to be followed. (2) In those adult patients in whom one may postulate the presence of islet tissue capable of stimulation, the larger amounts of carbohydrate may provide such. (3) By securing more calories by means of carbohydrate, it is possible to lower the amount of fat in the diet thereby, one hopes, lessening the chances of elevated blood cholesterol levels and atherosclerosis. The disadvantages of prescribing more than 200 gm. or at least more than 225 gm., of carbohydrate a day and of allowing "free diets" are: (1) If one gives much more than 200 gm. of carbohydrate a day, it is necessary to provide this in the form of more concentrated carbohydrate foods which are difficult for the patient to utilize. Most certainly if 250–300 gm. or more of carbohydrate are given daily, some sweets and pastries will be necessary because the bulk required to furnish this much carbohydrate by means of vegetables, fruit, and even of bread and potatoes is so great as to make the diet impractical for most adult patients. Sugar and sweets have the great disadvantage that, requiring a minimum of time foı digestion, they are absorbed into the blood stream so rapidly as to overwhelm the capacity of injected insulin to care for the situation. Consequently, hyperglycemia and glycosuria of unwanted degree are apt to occur. (2) Liberal or free diets may induce general laxity in treatment on the part of the patient. (3) With higher diets, there is a greater tendency for the patient to vary the intake of food from day to day, thus upsetting the balance between diet on the one hand and insulin and exercise on the other. (4) There is at least a theoretical possibility of overstimulation and overwork of the β-cells of a pancreas possessing little or no reserve. (5) There is a chance of producing obesity, acknowledged to be harmful to diabetics.

Much has been said and written regarding emotional and psychologic trauma supposedly induced by programs of careful control and dietary restriction, particularly in children and adolescents. However, such effects are not as great as often stated and even if undesirable attitudes do at times develop, one must weigh those consequences against the distressing and often disabling sequelae of poorly controlled diabetes such as retinopathy, neuropathy, and nephropathy.

PROTEIN. For the adult patient, enough protein must be supplied to provide at least ⅔ to 1 gm. per kilogram (2.2 lb.) of ideal body weight.

In actual practice, with most adults, 60 to 100 gm. of protein a day are desirable, with the actual amount gauged according to the age, weight, sex, occupation, complicating conditions, and dietary preferences of the person concerned. Certain young men doing heavy physical labor will require a liberal caloric intake, which may be provided in part by giving from 120 to 150 gm. of protein a day. With growing children care must be taken to keep the amount of protein at an optimal level; in the earlier age groups this may amount to as much as 2, 3 or even 4 gm. per kilogram of body weight per day.

FAT AND CALORIES. Having set the amount of carbohydrate and of protein at desired levels, the rest of the calories must be made up by fat. It is customary in clinical work to assume that each gram of carbohydrate and of protein provides 4 calories and that each gram of fat yields 9 calories. Therefore, if for a certain patient it is desired to give 200 gm. of carbohydrate, 100 gm. of protein, and a total caloric value of 2100 calories, then one prescribes 100 gm. of fat to bring the calories up to the desired level.

The optimal number of calories will depend upon the age, sex, occupation, body build, and the present body weight of the patient. In estimating the calories required, one keeps in mind those necessary for basal metabolism plus those required for physical activity. Because of great individual variation from person to person, one cannot predict with great accuracy the caloric needs of a given individual even though the basal metabolism (amount of oxygen consumed by the patient at rest and in the postabsorptive state) has been determined. With adult diabetic patients, it is common practice to allow 35 to 40 calories per kilogram of ideal body weight per day for a man engaged in moderate physical exercise and 30 calories per kilogram of body weight for those with light activity (2100 calories per day for a man weighing 70 kg., or 154 pounds). In this connection it is worthwhile to point out that caloric requirements given in published tables often err on the side of being too high. Most diabetic patients are middle-aged or elderly and, at least in the United States, relatively few are engaged in strenuous physical work. The average diabetic woman between 50 and 70 years of age whose activity consists of housework will maintain her weight and strength on a prescribed diet of 1600 to 1800 calories per day (about 25 calories per kilogram of body weight). In consequence, the amount of fat prescribed for most adult patients varies from 50 to 140 gm. a day and in most cases from 70 to 100 gm. a day.

Excess body weight should be carefully guarded against and if patients are already overweight, as is often the case, diets must be made low enough in calories to allow gradual yet steady reduction. If the

body weight is brought nearer to the ideal level, hyperglycemia and glycosuria become much easier to control and unless there are other complications, remain so unless the weight is subsequently regained. In planning diets designed for weight reduction, one must be particularly careful to insure an adequate intake of minerals and vitamins. For this reason the main part of the diet of overweight diabetic patients must be made up of lean meat (or its equivalent in other proteins), green vegetables, fruit, and skimmed milk.

The type of fat recommended to the patient deserves special consideration. Evidence has accumulated to suggest that the unsaturated fats may be less harmful than saturated fats as regards the production of hyperlipemia, hypercholesteremia, and vascular complications including atherosclerosis. By and large, unsaturated fats are liquid at room temperature and are of vegetable origin although there are certain exceptions (coconut oil is rich in saturated fats), and saturated fats are solid at room temperature and are of animal origin. The possible long-term harmful effects of saturated fats are, in the light of present knowledge, not sufficiently clear-cut to warrant any major change in the prescription of diabetic diets. However, there is enough information to make the following suggestions seem reasonable: (1) Diets high in fat (more than 100 gm. a day) should be avoided unless the caloric needs of the individual patient make such imperative. (2) The caloric content of diets should be kept low enough to avoid overnutrition and patients already overweight should receive diets low enough in calories to provide for gradual weight loss. (3) When there is a reasonable choice, fat of vegetable origin should be chosen rather than that of animal origin. Thus vegetable oils would appear to be preferable to lard and animal fat.

THE DIETARY PRESCRIPTION

The diet prescribed at the beginning of treatment should be somewhat lower than that which will be advised after regulation has been completed. This facilitates initial lowering of the blood sugar and clearing of glycosuria. However, such restriction need not be marked and most adult patients can with benefit be started on a diet containing at least 130–150 gm. of carbohydrate, 60–70 gm. of protein, and 60–80 gm. of fat a day. After one or more days, the amount of food may be altered in one or more of its components as the individual situation indicates.

In adult patients in whom the diabetic condition seems "mild," as suggested by only moderate hyperglycemia and glycosuria despite a

previously unrestricted diet, it may suffice to prescribe simply avoidance of sweets and pastries, substituting as desserts average servings of fresh or water-packed fruit, and decrease in the amount of other carbohydrate-rich food, as by the restriction of bread to one slice at a meal. One may either omit potato, rice, corn, shell beans, and macaroni or limit to one moderate serving daily any one of these foods. A diet of this type might, in addition, include one egg, bacon, cereal, and fruit for breakfast, and average amounts of meats and vegetables (other than those just mentioned) at the other two meals, together with one-half to one pint of milk, one-fourth pint of cream, and one ounce of butter or margarine daily. Such a diet furnishes approximately 140 to 170 gm. of carbohydrate a day. If a patient of this type is obese, the amount of carbohydrate allowed would be even less and the allowance of fat severely curtailed.

However, in most diabetic patients, much more specific and detailed instruction is imperative. The patient should be told not only what to avoid but also what to eat and the quantities of the various foods should be clearly stated in writing. The average patient wishes to co-operate and will gladly do so but to do this he must have instructions that are definite, simple, and easy to understand.

So far in this discussion there has been no direct statement as to how to arrive at the initial dietary prescription. This is a natural consequence of the fact that experience throughout the world has shown that the best plan is not to pick from a table or textbook a dietary prescription based on theoretical requirements but rather to select as an initial diet one which seems reasonable for the patient concerned and which is in keeping with best of current thought as regards composition. From then on, adjustments made in the basic diet become a matter of trial and error. At first thought this appears totally unscientific, but actually the experience of many clinicians throughout the world over decades of time has shown it to be the most practical method At the present time the writer and his colleagues use as a basic diet for the initiation of treatment in many adult patients one that calls for approximately 150 gm. of carbohydrate, 70 gm. of protein, and 60–80 gm. of fat. As previously stated, one has in mind that the final diet after regulation has been accomplished would provide for 150–200 gm. of carbohydrate, 60–100 gm. of protein, and 60–100 gm. of fat, furnishing about 1400–2100 calories per day. It must be understood that these are average figures and that considerably different values may be necessary for each of the dietary components, depending upon the age, sex, occupation, body build, and present body weight of the individual patient.

DISTRIBUTION OF FOOD

The division of the carbohydrate during the day is commonly set at one fifth for breakfast, two fifths for the noon meal, and two fifths for the evening meal, although variations from this are permissible to meet special conditions encountered in any individual patient. In order to provide automatic protection against hypoglycemic attacks because of insulin, between-meal snacks should be arranged. The afternoon snack is particularly desirable in patients receiving NPH or lente insulin and in the form of crackers and milk or fruit should provide 15–20 gm. of carbohydrate a day. Likewise a bedtime snack of 10–20 gm. of carbohydrate should be given regularly. In certain patients receiving crystalline insulin before breakfast, a mid-morning snack of 10 gm. of carbohydrate is in order. These snacks form parts of and not additions to the basic diet.

INSTRUCTION OF THE PATIENT

From the very start of treatment patients and their families should receive simple and yet detailed instruction in matters of diet. They should be sufficiently well acquainted with food values to be able to substitute one food for another so that, whether at home or away from home, they may select with reasonable accuracy the amount of food called for on their particular diet. To the extent to which their intelligence and ability to learn will allow, they should be taught the carbohydrate, protein, fat, and caloric content of various foods and how to calculate diets. There is abundant evidence to indicate that those patients who are trained in this fashion are the ones who are most apt over years of time to maintain good control of diabetes with the reward of prevention or postponement of late complications. The various methods of selection of food may be listed as follows:

ESTIMATION OF FOOD. Simple avoidance of sweets and pastries and restriction of other carbohydrate-rich foods and, if indicated, restriction of foods rich in fat.

MEASUREMENT OF FOOD. This popular technique calls for the measurement of food by means of teaspoons, tablespoons, and measuring cups graduated in ounces. For foods such as meat, the amount is gauged by means of standard wooden blocks or similar devices.

WEIGHING OF FOOD. This is the method of choice and is the one preferred by the writer and his associates. Gram scales with a movable dial that allow for the taring of the container permit rapid weighing even by the inexperienced. With intelligent, co-operative patients, the

weighing of food at the initiation of treatment is an invaluable exercise. One must freely acknowledge that the carbohydrate, protein, and fat content of different samples of food may vary from one to another considerably and that the actual values for a given serving of food may differ somewhat from the average values found in published tables. However, if instruction in weighing is given, there is more likelihood that the quantities of food actually eaten will be closer to the desired amount than if the diet is merely estimated or is selected by household measures. Instruction in weighing serves to acquaint the patient and his

TABLE 33-1. CARBOHYDRATE, PROTEIN, AND FAT CONTENT AND CALORIC VALUE OF 30 GRAMS (1 OUNCE) OF CERTAIN FOODS*

30 Grams (1 oz.) contain approximately	*Carbohydrate Gram*	*Protein Gram*	*Fat Gram*	*Calories*
Bread, 1 slice (24 Gm.)	12	2	0	56
Oatmeal, large portion	20	5	2	118
Crackers	20	3	2	110
Vegetables, 3%†	1	0.5	0	6
Vegetables, 6%	2	0 5	0	10
Potato	6	1	0	28
Milk	1 5	1	1	19
Egg, 1	0	6	6	78
Meat, lean	0	7	5	73
Chicken, lean	0	8	3	59
Fish, fat-free	0	6	0	24
Cheese	0	8	10	122
Bacon	0	5	15	155
Cream, 20% (light)	1	1	6	62
Cream, 40% (heavy)	1	1	12	116
Butter or margarine	0	0	25	225

* Adapted from a table by Joslin (1).
† With most patients these foods of low carbohydrate content may be allowed freely

family with simple scientific procedures and principles. One must hasten to add, however, that weighing should be regarded as a means to an end rather than as an end in itself. It should be carried out for 4 to 6 weeks or for such time as is necessary for the eye and hand to judge various types of food with reasonable accuracy. Then one returns to weighing for short periods from time to time for refresher experience or when some new food is eaten.

The education of the patient as regards the nature of diabetes, urine testing, diet, insulin, oral hypoglycemic agents, and complications must begin with *the first visit and must be a continuing process.* Such instruction should be carried out at personal interviews with the physician,

nurse, and dietitian, and in classes. The association with other diabetic patients is most helpful. Over the years we have been impressed by the value of having a wing or floor of the hospital in which ambulatory patients may be housed and receive an intensive course of instruction for a week or less while at the same time receiving weighed diets, qualitative tests for sugar in urine before meals, determination of the grams

TABLE 33-2. VEGETABLES, FRESH OR CANNED (WATER-PACKED) AND CERTAIN RELATED FOODS ARRANGED IN GROUPS ACCORDING TO THEIR APPROXIMATE CARBOHYDRATE CONTENT*

3 Per cent	
Lettuce	Tomatoes
Cucumbers	Radishes
Spinach	Water cress
Asparagus	Snap beans
Celery	Cauliflower
Mushrooms	Cabbage
Rhubarb	Eggplant
Sauerkraut	Broccoli
Endive	Green peppers
Swiss chard	Kohl rabi
Beet or other greens	Kale
	Summer squash

6 Per cent	*20 Per cent*
Turnip	Potatoes
Carrots	Shell beans
Okra	Lima beans
Pumpkin	Corn
Onions	Boiled rice
Squash	Boiled macaroni
Brussels sprouts	
Beets	
Green peas	

* Adapted from a table by Joslin (1).

of sugar in the urine each 24 hours, and appropriate tests of the blood sugar not only before breakfast but before the other two meals as well. Patients undergoing this type of hospitalization benefit greatly and the knowledge and inspiration received will make a great impression on them that will linger for a long time. At intervals they should be brought back for renewed training. Hospitalization for ambulatory patients requiring no special nursing care may be provided at lower cost. If facilities are not available for this type of instructional program, then a

plan for teaching on an outpatient basis should be provided. In perhaps no other disease is it so imperative that the patient learn as much as he can about the nature and treatment of his condition and the importance of restoring physiological conditions as nearly as possible.

CALCULATION OF DIETS

In the calculation of the carbohydrate, protein, and fat content of food, the amounts selected as units may be either 30-gm. (one ounce) or 100-gm. portions. Either method is satisfactory. The latter conforms more nearly to methods used in scientific work but the former has the

TABLE 33-3. AMOUNTS OF VARIOUS FRUITS (FRESH OR WATER-PACKED) REQUIRED TO YIELD 10 GRAMS OF CARBOHYDRATE*

Fruit	*Grams*
Grapefruit	150
Strawberries	150
Watermelon	150
Cantaloupe	150
Blackberries	120
Orange	100
Pears	90
Peaches	90
Apricots	80
Raspberries	80
Plums	80
Pineapple	70
Apple	70
Honeydew melon	70
Blueberries	70
Cherries	60
Banana	50
Prunes (cooked)	50

* *Adapted from a* table by Joslin (1)

great advantage that the patient is able to bridge the gap between the English and metric systems of weights and measures more easily. In Table 33-1 are given figures for various foods Although only 16 items are listed, these when taken in connection with their equivalents will furnish values for most of the foods commonly eaten. The equivalents for potato as well as a classification of vegetables according to their approximate carbohydrate content are given in Table 33-2. Finally, in Table 33-3 is given the amount of various fruits, fresh or water-packed, yielding 10 gm. of carbohydrate. Using these three simple tables, it is easy to carry out the calculation of any diet commonly prescribed for

TABLE 33-4. DIET SUITABLE FOR CERTAIN ADULT DIABETIC PATIENTS, SHOWING CALCULATIONS IN DETAIL*

	Household measure	*Grams*	*Carbo-hydrate*	*Protein*	*Fat*	*Calories*
Breakfast						
Orange juice	small glass	120	12	0	0	48
Egg	One	. .	0	6	6	78
Oatmeal (cooked)	small serving	120	10	3	1	61
Cream, 20%	2 ounces	60	2	2	12	124
Bread	1½ slice	36	18	3	0	84
Butter	1 teasp.	5	0	0	4	36
	Total		42	14	23	431
Lunch						
Lean meat	medium serving	90	0	21	15	219
Vegetables, 3%	freely	150+	5	2	0	28
Vegetables, 6%	1 med. serving	75	5	1	0	24
Bread	1 slice	24	12	2	0	56
Butter	2 teasp.	10	0	0	8	72
Cream, 20%	1 ounce	30	1	1	6	62
Orange	1 medium	150	15	0	0	60
Milk	½ pint	240	12	8	8	152
	Total		50	35	37	673
Afternoon snack						
Orange	1 medium	150	15	0	0	60
Supper						
Lean meat	medium serving	90	0	21	15	219
Vegetables, 3%	freely	150+	5	2	0	28
Vegetables, 6%	1 med. serving	75	5	1	0	24
Potato	1 medium	90	18	3	0	84
Bread	1 slice	24	12	2	0	56
Butter	2 teasp	10	0	0	8	72
Cream, 20%	1 ounce	30	1	1	6	62
Orange	1 medium	150	15	0	0	60
	Total		56	30	29	605
Bedtime snack						
Milk	½ pint	240	12	8	8	152
Grand total			175	87	97	1921

* In this diet, for the sake of simplicity, foods are expressed in general terms. Instead of oatmeal, an equivalent amount of any cereal, dry or cooked, may be used In place of orange, equivalent amounts of other fresh fruit may be substituted (see Table 33-3). In place of meat one may use fish, fowl, cheese, or eggs in quantities yielding approximately the same amounts of protein and fat

diabetic patients. In Table 33-4 is shown such a detailed calculation for a diet providing approximately 175 gm. of carbohydrate, 87 gm. of protein, 97 gm. of fat, and 1921 calories.

MEAL PLANNING WITH EXCHANGE LISTS

Several years ago committees of the American Diabetes Association and the American Dietetic Association, in co-operation with the Diabetes Branch of the U.S. Public Health Service, prepared a booklet entitled "Meal Planning with Exchange Lists." This has been widely used and thousands of copies have been distributed. Although in the opinion of the writer this system of food measurement and substitution is not as satisfactory as that of weighing and calculation of diets, there can be no doubt but that its use has improved greatly the standards of dietary treatment throughout the country. In this plan, foods are divided into six groups called exchange lists. Each food, in the amount stated, contains about the same amount of carbohydrate, protein, or fat as any other food in that list. The lists and the standard type and amount of food are shown in Table 33-5.

TABLE 33-5. EXCHANGE LISTS

List	*Exchange lists*	*Food type*	*Amount*	*CHO*	*P*	*F*	*Calories*
1	Milk	Whole milk	1 cup	12	8	10	170
2A	Vegetable		Freely				
2B	Vegetables		½ cup	7	2	0	35
3	Fruit	Orange	1 small	10	0	0	40
4	Bread	Bread	1 slice	15	2	0	70
5	Meat	Meat	1 ounce	0	7	5	75
6	Fat	Butter	1 teasp.	0	0	5	45

The physician directs the patient as to the number of exchanges to take from the various lists for the three main meals and between-meal snacks. For convenience in prescribing, six different meal plans have been printed, each on a separate leaflet. Meal plan 1 provides 125 gm. of carbohydrate, 60 gm. of protein, 50 gm. of fat, and 1200 calories. Meal plan 6 provides 250 gm. of carbohydrate, 100 gm. of protein, 130 gm of fat, and 2600 calories. The other meal plans provide for intermediate amounts of the various food components and calories. The booklets together with the meal plans may be obtained from the American Diabetes Association, Inc., 1 East 45th Street, New York 17, N.Y., or from the American Dietetic Association, 620 North Michigan Avenue, Chicago 11, Illinois.

As has been stated, the system of meal planning with exchange lists has proved its worth. However, to use the plan most advantageously, the following points should be kept in mind: (1) The physician should know what he is prescribing. To know that the patient is receiving food called for on ADA Meal Plan 3 and perhaps to know that this provides about 1800 calories is not enough. The physician should know that this meal plan furnishes approximately 180 gm. of carbohydrate, 80 gm. of protein, 80 gm. of fat, and 1800 calories per day. Furthermore, by sample calculation of this or similar diets, he should know what foods actually make up the diet. (2) The diabetic patient himself should receive as much detailed information regarding his diet as his intelligence and interest will allow.

VITAMINS AND MINERALS

In the planning of any special diet, care must be taken to provide adequate amounts of vitamins and minerals. Fortunately, the diabetic diet as usually outlined assures adequacy in this regard. It is worthwhile emphasizing, however, that the greater freedom made possible by higher carbohydrate diets should not lead to the forsaking of fruits and green vegetables. These should be prescribed in adequate amounts and patients taught to like them. In certain patients the supplementing of the diet with appropriate amounts of vitamin B or other vitamins may be indicated and the physician must be alert to needs in this regard. As for minerals, care must be taken to provide enough calcium, not only for children but also for older patients. Since the important source of calcium is dairy products, including milk, cream, and cheese, these foods should be supplied in adequate amounts. When diets low in fat are prescribed, the calcium allowance may suffer because of the exclusion of cream, cheese, and whole milk; to avoid this, skimmed milk should be used.

SPECIAL FOODS

The most useful of the special foods for diabetic patients are the water-packed fruits and vegetables, which now can be purchased at popular prices in grocery stores. Water-packed fruit sweetened with saccharine or Sucaryl is available, if desired. Calorie-free carbonated beverages are permissible. Certain hard candies prepared for use by diabetics are sufficiently low in sugar and calories as to provide very little upset in the diabetic condition. However, the use of "dietetic" or "diabetic" soft candy, chocolates, cookies, etc., is to be discouraged. It is true that these products are prepared without the use of actual sugar

but they do contain basic components which have food value. If the patient would substitute such foods for articles called for on his diet, no great harm would be done except that the special foods cater more to the taste or whim of the patient than to his nutritional needs. Furthermore, the patient is apt to believe that such special foods may be added to his diet and eaten over and above the food regularly prescribed. "Diabetic" ice cream is made without the use of actual sugar but other ingredients required for its manufacture do have food value. If the patient takes this type of ice cream, he should use it in such quantity as allowed in place of his regular dessert. As for ordinary commercial ice cream, the carbohydrate content is high, averaging about 20 per cent. It is, therefore, not truly a suitable food for inclusion in a diabetic diet although the common custom is to allow a small serving, 50–75 gm., once or twice a week.

ALCOHOL

Although alcohol yields in the body 7 calories per gram, it can scarcely be classed as a true food. Among the various reasons why diabetic patients do well to avoid alcohol are the following: (1) Those beverages with a low alcohol content, such as beer and certain wines, contain significant amounts of carbohydrate (a 12-oz. bottle or can of beer contains about as much carbohydrate as a slice of bread), so that taking them uses up a definite amount of the day's allowance of food. (2) Although beverages with a high alcoholic content such as whisky, brandy, rum, and gin do not significantly increase glycosuria in the amounts ordinarily taken, social custom includes usually the taking of some food along with the drinks and this may definitely alter control of the diabetic condition. (3) The patient under insulin treatment makes himself liable to the mistake easily made by the laity of confusing with true intoxication an insulin reaction in a person who has had a little alcohol.

DIET FOR DAYS OF ACUTE ILLNESS

During days of acute illness, following surgery, recovery from acidosis, etc., *the diet may be modified and concentrated to suit the needs of the* individual patient. When only fluids can be tolerated, these may be supplied in the form of orange or other fruit juices, ginger ale, milk, etc., in small amounts at frequent intervals providing at least 100 and preferably 150 gm. of carbohydrate per day. *When soft food can be borne,* a diet such as that outlined in Table 33-6 may be given.

TABLE 33-6. EXAMPLE OF A CONCENTRATED DIET FOR A DAY OF MINOR ILLNESS

	Household measure	*Grams*	*Carbohydrate*	*Protein*	*Fat*	*Calories*
Breakfast						
Orange juice	3½ ounces	100	10	0	0	40
Egg	One	. . .	0	6	6	78
Bread	1 slice	21	12	2	0	56
Butter	1 teasp.	5	0	0	4	36
Milk	6 ounces	180	9	6	6	114
		Total	31	14	16	324
Forenoon snack						
Cracker, 2½ in. sq.	One	. . .	5	1	1	33
Milk	4 ounces	120	6	4	4	76
		Total	11	5	5	109
Lunch						
Oatmeal (cooked)	average serving	240	20	5	2	118
Milk	6 ounces	180	9	6	6	114
Cream, 20%	2 ounces	60	2	2	12	124
Bread	1 slice	21	12	2	0	56
Butter	1 teasp.	5	0	0	4	36
Orange juice	3½ ounces	100	10	0	0	40
		Total	53	15	24	488
Afternoon snack						
Crackers, 2½ in. sq.	Two	.	10	1	1	53
Milk	6 ounces	180	9	6	6	114
		Total	19	7	7	167
Supper						
Egg	One	. . .	0	6	6	78
Bread	1 slice	21	12	2	0	56
Butter	1 teasp.	5	0	0	4	36
Milk	6 ounces	180	9	6	6	114
Orange juice	3½ ounces	100	10	0	0	40
		Total	31	14	16	324
Bedtime snack						
Crackers, 2½ in. sq.	Two	.	10	1	1	53
Milk	6 ounces	180	9	6	6	114
		Total	19	7	7	167
Grand total			164	62	75	1579

It is common to prescribe a meal at noon and night of essentially the same type and composition. However, this is by no means necessary and either in the hospital or in the home, adjustments can be made so as to provide a more concentrated meal or lunch in the middle of the day.

TABLE 33-7. SUGGESTIONS FOR LUNCHES CONTAINING APPROXIMATELY 50 GRAMS OF CARBOHYDRATE

Food	*Household measure*	*Grams*	*Carbo-hydrate*	*Pro-tein*	*Fat*	*Calories*
I.						
Bread	2 slices	48	24	5	0	116
Meat	small serving	75	0	20	13	197
Lettuce	as desired	. . .	. . .	. . .	. . .	. . .
Butter	1 teasp.	5	0	0	4	36
Milk	½ pint	240	12	8	8	152
Apple	1 small	105	15	0	0	60
Total			51	33	25	561
II.						
Bread	2 slices	48	24	5	0	116
American cheese	1½ ounces	45	0	12	16	192
Lettuce	as desired	. . .	. . .	. . .	. . .	. . .
Butter	1 teasp.	5	0	0	4	36
Egg	One	. . .	0	6	6	78
Milk	½ pint	240	12	8	8	152
Orange	1 medium	150	15	0	0	60
Total			51	31	30	598
III.						
Bread	2 slices	48	24	5	0	116
Choice of filling						
Crabmeat, tuna, salmon	2½ ounces	75	0	20	13	197
Lettuce	as desired	. . .	. . .	. . .	. . .	. . .
Mayonnaise	1 tablespoon	15	0	0	12	108
Milk	½ pint	240	12	8	8	152
Banana	1 small	75	15	0	0	60
Total			51	33	33	633

This is particularly useful for children at school and for other patients who must have the noon meal away from home. The lunches which can be prescribed do not differ materially from those used by people in general. In Table 33-7 are shown examples of lunches providing approximately 50 gm. of carbohydrate

EXERCISE

In patients receiving insulin or in those persons with mild diabetes who retain a considerable capacity for endogenous insulin production, physical activity accentuates greatly the blood sugar lowering effect of insulin. Diabetic patients soon come to know that on days of increased physical activity they are more liable to insulin reactions. Children with diabetes who come from a life at school to a much more active life in a summer camp often can have their condition much better controlled with smaller doses of insulin, despite liberal diets. Patients should be taught to take advantage of this beneficial effect of physical activity and to engage in moderate exercise regularly. They should be taught also to take small amounts of additional food on days during which it can be anticipated that unusual exercise will be had.

It follows from the above that, in the period of regulation of diabetes in a hospital, patients should be out of bed and up and around as much as their physical condition will allow. Otherwise the amount of insulin found necessary under the quiet conditions of the hospital will prove excessive when the patient returns home. Even with patients confined to bed, often mild forms of exercise may be possible and, in addition to the beneficial effect upon the diabetes, muscular tone may be preserved.

SPECIAL EXAMINATIONS

Not only should the patient with diabetes be seen regularly by the physician and the diabetic status evaluated but the opportunity should be seized at these intervals, which may vary from 1 to 6 months depending upon the needs of the patient, to carry out interval histories and physical examinations. The care of the diabetic patient involves much more than the regulation of the diet and the insulin dose. It involves the care of the whole person and attention to his various physical and emotional problems.

In the physical examination, particularly of the adult patient, especial attention should be given to the feet. The feet should be examined routinely for evidences of epidermophytosis or other minor infections. Corns and calluses should be noted, if present, and the patient referred to a competent chiropodist who is acquainted with the problems of diabetes. The peripheral pulses, dorsalis *pedis*, *posterior* tibial, and popliteal should be sought for and the results of palpation recorded not only for present information but for future reference. Due regard

should be had for the appearance, temperature, and nutritional status of the feet.

The mouth should be regularly examined and the patient referred to a competent dentist for the treatment of any conditions for which there is need. The ophthalmoscope should be used quite as much as the stethoscope and the earliest indication of ocular abnormalities noted. X-rays of the chest should be had at appropriate intervals despite the fact that the incidence of pulmonary tuberculosis in diabetic patients is fortunately no longer as high as it once was. Because of the relatively high incidence of urinary tract infections and nephropathy, examinations of the urine for albumin and for abnormalities in the sediment should be made at each visit.

Perhaps more than any other class of patients, those with diabetes visit their physicians oftener and over a longer period of time. There is afforded consequently an ideal opportunity for the early detection of coexisting diseases such as tuberculosis, cancer, heart disease, etc. The physician will do well to be alive to this possibility and the attendant responsibility.

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Chapter 34

TREATMENT WITH INSULIN

Arthur R. Colwell, Sr.

The use of insulin in the routine treatment of diabetes mellitus will be discussed in this section. It will be assumed that dietary factors are properly appreciated (Chap. 33), that the supplementary use of insulin for acute emergencies is discussed adequately elsewhere (Chaps. 36 and 37) and that the comparative indications for insulin and orally effective blood sugar lowering agents are clear (Chap. 35).

OBJECTIVES IN TREATMENT

All therapy in diabetes mellitus presumes that there are advantages in restoring to normal as many clinical features of the disorder as possible. Laboratory indexes most readily available are blood and urine sugar values. By no means is diabetes a disorder of glucose metabolism alone. Nevertheless, there is general agreement that treatment is adequate when, among other things, *blood and urine glucose values are kept as nearly normal as is possible without some greater penalty* such as severe or frequent insulin shock or intolerable inconvenience. When food intake is optimal and no acute complication interferes it is a fact that health is then maintained and complications are prevented or de-

layed in spite of the existence of diabetes. It is this that is meant by the term "good control."

With the use of modern methods it is possible to maintain approximately normal blood and urine sugar values continuously in some 80 per cent to 90 per cent of all diabetic patients. The fact that actual experience is not as favorable can be attributed to inadequate attention on the part of either the physician or the patient or both. The minority of diabetic patients who cannot maintain good control without intolerable inconvenience (labile or "brittle" diabetes: see below) can escape immediate harm by holding hyperglycemia and glycosuria at minimum levels compatible with freedom from insulin shock. This policy is a matter of expediency. It must not lead to carelessness in the great majority of diabetics in whom it is unnecessary.

Known penalties of poor control by such standards include:

1. Discomfort, particularly fatigue, but sometimes thirst, polyuria, pruritus, and weight loss as well.
2. Premature irreversible damage to the β-cells in the islets of Langerhans leading to permanent increases in severity of the diabetic process.
3. Infections, particularly of the skin and urinary tract.
4. Over long periods of time, probably vascular (including renal and retinal) disease.

INDICATIONS FOR INSULIN

When an essentially normal metabolic and nutritional state can be maintained without the use of insulin, nothing more is necessary. When it cannot, insulin should be used. The choice of available preparations, of dosage, and frequency of administration depend upon the needs of the individual subject. When food intake is constant and no complications exist, these can be determined by frequent blood and urine glucose determinations. In general, the more severe the disorder of glucose metabolism the greater is the need for insulin and the more skill and discrimination are required for its effective use. Severity is not necessarily proportional to the amount of insulin required nor to the magnitude of the blood and urine glucose values. It is much more nearly proportional to the ease with which poor control occurs as a result of improper food intake, errors in insulin dosage, and intercurrent illness (2).

Without extravagance in eating it is possible for fully 50 per cent of all diabetics to keep well controlled and not use insulin. Orally effective

hypoglycemic agents may increase this number substantially but probably not to the extent of the 80 per cent sometimes stated (see Chap. 35).

In order of increasing severity, diabetes mellitus in which insulin is

TABLE 34-1. TYPES OF DIABETES MELLITUS WHICH REQUIRE INSULIN FOR GOOD CONTROL: COMMON CLINICAL CHARACTERISTICS AND APPROPRIATE TECHNIQUES OF USING INSULIN SHOWN FOR EACH

Severity	*Common clinical characteristics*	*Most appropriate insulin technique*
Moderate	Stability of behavior Onset in adult and/or early in course of juvenile diabetes Ketoacidosis infrequent Not sensitive to insulin	Any depot insulin once daily in moderate dosage Protamine zinc and ultralente insulins useful only here (Oral hypoglycemic agents sometimes effective)
Severe	Some children and juveniles After long duration in some adult cases Sugar balance shifts easily Moderately sensitive to insulin Good control possible with proper use of insulin Lose weight easily	Intermediate-acting depot insulins* once daily before breakfast, usually in fairly large dosage
Labile ("brittle" or "unstable")	Most children and juveniles Some adults, especially those who are thin During chronic complications, especially infections Acidosis on slight provocation Sensitive to insulin, frequent unexpected shock	Intermediate-acting insulins* twice daily Intermittent glycosuria unavoidable Avoid insulin shock Interval feedings Watch for ketonuria
Acute complications	Acute infections Ketoacidosis of any degree Trauma Surgery Other acute illness	Unmodified insulin in solution Frequent dosage: every few hours Frequent testing to determine response Depot insulins unreliable

* Globin, NPH, lente, and mixtures of unmodified and protamine zinc insulin

required can be classified as in Table 34-1. Common clinical characteristics and the *most useful insulin techniques* are shown for each class. Acute complications of and with diabetes are included for completeness (see also Chaps. 36 and 37).

TIMING OF ACTION AND USAGE OF VARIOUS FORMS OF INSULIN

No matter the form in which they are marketed, all varieties of insulin have two things in common: (1) for practical purposes they are effective *only when given parenterally;* (2) their action, dependent entirely upon their hormone (insulin) content, is determined by the rate at which in-

TABLE 34-2. COMPOSITION OF AMERICAN INSULINS

Type	*Units per ml.*	*Form*	*pH*	*Mg. per 100 units*		*Buffer*
				Zinc	*Protein*	
Long-acting						
Protamine zinc	40 or 80	amorphous suspension	7.1 to 7.4	0.2 to 0.25	Protamine 1.0 to 1.5	sodium *phosphate*
Ultralente	40 or 80	crystalline suspension	7.1 to 7.5	0.2 to 0.25	. .	sodium acetate
Intermediate						
2:1 Mixture	80	amorphous suspension	6.0 to 6.4	0.07 to 0.11	Protamine 0.33 to 0.5	sodium phosphate
Globin	40 or 80	solution	3.4 to 3.8	0.25 to 0.35	Beef globin 3.6 to 4.0	.
Isophane (NPH)	40 or 80	crystalline suspension	7.1 to 7.4	0.016 to 0.04	Protamine 0.3 to 0.6	sodium phosphate
Lente	40 or 80	30% amorphous and 70% crystalline suspension	7.1 to 7.5	0.2 to 0.25	.	sodium acetate
Prompt-acting						
Semilente	40 or 80	amorphous suspension	7.1 to 7.5	0.2 to 0.25	.	sodium acetate
Zinc-insulin Crystals	40, 80, 100 or 500	solution	2.5 to 3.5	0.01 to 0.04	.	

sulin is released and distributed after parenteral injection. In practice, parenteral means subcutaneously (rarely intravenously) and rate of insulin release means the rate at which the subcutaneous deposit gives up its hormone by absorption in the case of plain solutions of insulins and by enzymatic dissociation of the hormone from its insoluble complex in the case of the various "depot" insulins.*

All forms of insulin currently available† can be put into one of three

* Although one of the lente insulins is injected as a suspension, its amorphous crystals (semilente) quickly go into solution after injection, so its action is like that of insulin solutions. Conversely, although globin insulin is in solution as marketed, it is precipitated on neutralization after injection, so its timing is like those of the depot insulins.

† Insulins in variety are manufactured and available in other countries. Their *timing of action has not been determined precisely in most instances.* Many are not available in this country, so only American and Canadian products will be included in this discussion. British and Scandinavian products are similar, although not identical.

categories, each with a fairly distinctive rate of insulin release and hence fairly distinctive speed, intensity, and duration of action after injection as a single subcutaneous dose. Their composition and physical characteristics are shown in Table 34-2. Inasmuch as the needs of various types of diabetes are met by different kinds of insulin with different timing characteristics, it will be profitable to review the typical action of each group, as a prelude in each case, to their specific application in therapy. As a general rule the less severe the diabetic state the less important is the need for insulin with prompt and intense action. Accordingly (and in conformity with the scheme outlined in Table 34-1), slower-acting insulins and milder forms of diabetes will be discussed first and prompt-acting insulins and severe diabetes later.

Slow-Acting Insulins: Moderate Diabetes

The first depot insulin to become generally available was *protamine zinc insulin*, a Danish product (6), prepared by precipitating insulin in firm combination with protamine and zinc so that a suspension is formed in a neutral buffer solution.

After subcutaneous injection a single dose of this compound releases its insulin slowly, as shown in Figure 34-1 (2). Action in diabetic humans is measurable in a few hours, with few exceptions reaching its peak in 16 to 32 hours—depending upon the size of the dose and the individual treated. The effect of a single dose is never very intense, but it continues for 24 to 72 hours, again depending upon the dosage and individual.

Ultralente insulin, also originating in Denmark (7), is a crystalline suspension of insulin with zinc in an acetate buffer. Unlike all depot insulins except the other lente preparations, it contains no protein precipitant but depends for its insolubility upon (1) its high zinc content; (2) the use of acetate, not phosphate, as the buffer; and (3) the crystalline form of its precipitate. Its action after subcutaneous injection is slow, comparable to protamine zinc insulin, with which its indications for usage are virtually identical.

The time-action of both of these preparations can be speeded up by admixture with appropriate prompt-acting analogues. Solution of zinc insulin crystals should be used with protamine zinc insulin and semilente with ultralente. Intermediate-acting modifications are thus produced: they will be discussed later.

Because of their slow and sustained rate of action, protamine zinc and ultralente insulins never need to be injected more often than once daily, almost always in the morning before breakfast. The effects of daily injections overlap each other with the result that the final response

to a given daily dose cannot be determined for several days. Then it is seen most directly in the blood glucose value in fasting. For the same reason, when one of the slow-acting insulins is used to replace unmodified insulin used previously the dosage of insulin should be reduced slowly, over a period of two days or so.

Stable diabetes of moderate severity traditionally has been treated with protamine zinc insulin. Ultralente insulin can be used for this purpose just as well. Injections are given only once daily, before breakfast as a rule. Seldom should a dosage of 30 units be exceeded: when urine glucose tests suggest need for more, intermediate insulins usually are indicated. Less than 10 units daily is rarely indicated. The time interval between the injection and the breakfast is not important, but the meal should not be delayed more than an hour or so beyond the usual time because of the risk of hypoglycemia from previous days' injections.

The proper dose for the individual patient is best determined by the level of the blood glucose before breakfast. When that is normal but glycosuria appears after a meal, either the meal is too large or an intermediate-acting insulin is indicated.

The precise value of meals is not important except that the breakfast ordinarily should not be large and a bedtime feeding usually is wise in anticipation of the long overnight period without food. Shifting carbohydrate from one time of day to another, as indicated by urine and blood glucose tests and insulin reactions, is more effective than arbitrary rules governing meal size.

The slow-acting insulins tend to (1) act unpredictably from dose to dose, probably because of their low solubility; (2) permit waves of hyperglycemia and glycosuria postprandially; and (3) in large dosage cause insulin shock, which may be severe and prolonged, during sleep or exercise (3). In severe forms of diabetes, insulins with intermediate rates of action are replacing those with slow action. Even in stable diabetes where slow-acting insulins are satisfactory, intermediate preparations are equally so.

Intermediate Insulins: Severe Diabetes

There are four kinds of insulin in common use in this country with speed and intensity of action intermediate between ordinary solutions of insulin at one extreme and protamine zinc insulin at the other. In order of appearance historically they are as follows.

Mixtures of insulin with protamine zinc insulin. Soon after its introduction in 1936, it was found that protamine zinc insulin allowed blood and urine glucose levels to rise too high after meals and to fall too low during fasting. In mild diabetes this did not occur, but in more severe

diabetes it led to the simultaneous use of protamine zinc and ordinary insulin, first by injection separately and then by admixture. But small amounts of added insulin were precipitated by the excess of protamine in protamine zinc insulin, so no acceleration of its action resulted until at least as much insulin as protamine zinc insulin was used. Our studies showed the most useful proportions to be from two to three parts of unmodified insulin to one part of protamine zinc insulin, thoroughly mixed in the syringe or ampoule, and preferably in the U-80 concentration (3). Typical time and intensity of action after a single large

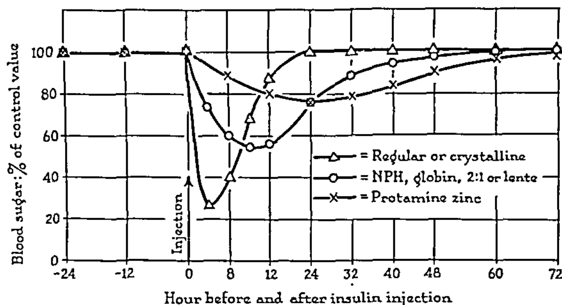


FIG. 34-1. Diagrammatic curves of average response of blood glucose in diabetic patients to single large subcutaneous injections of unmodified insulin (triangles), any of the intermediate insulins in common use (open circles) and protamine zinc insulin (crosses).

subcutaneous dose in diabetes of average severity is shown by the intermediate curve in Figure 34-1, compared with unmodified and protamine zinc insulin.

Globin insulin with zinc appeared in 1939 as a result of the work of Reiner, Searle, and Lang. It has been used widely in Great Britain. It contains 3 8 mg. beef globin and 0.3 mg. zinc per 100 units insulin. At its pH of 3.6 it is in solution, but it precipitates between pH 5 and 8 with optimum flocculation at pH 6.1. Thus, after subcutaneous injection it obviously precipitates like other depot insulins.

The timing of globin insulin is almost indistinguishable from that of the other intermediate preparations (see Fig. 34-1), but most investi-

gators judge it to act a little faster and wear off a little sooner than the intermediate protamine modifications in common use, namely, the 2:1 mixture and NPH, and lente insulin.

*Isophane (NPH)** *insulin is a suspension of crystals prepared by* using the smallest amount of protamine that will precipitate insulin completely at pH 7.2 in the presence of 0.03 mg. zinc per 100 units (9, 10). The crystals are tetragonal, have shiny faces and sharp edges, and

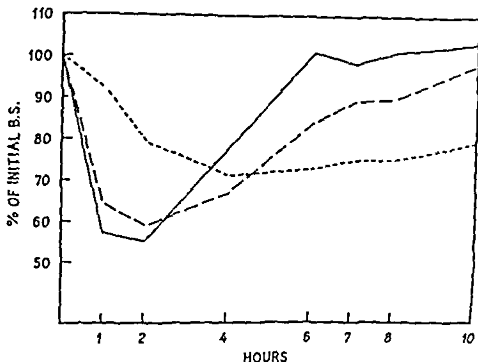


FIG. 34-2. Cross-over retardation test. Average blood glucose response in 26 rabbits, each given 3 units of ordinary insulin (solid line), semilente insulin (long dashes), and ultralente insulin (short dashes). (By permission of K. Hallas-Møller, Ph.D., Copenhagen.)

the phosphate buffer in which they are suspended contains practically no free insulin nor protamine. Thus, the suspension is stable and uniform in action, and ordinary insulin may be added to it in moderate amounts (10 per cent to 20 per cent) without change in action, for short periods, of either preparation.

NPH insulin was designed to reproduce the timing of action of the commonly used 2:1 mixture (2 insulin with 1 protamine insulin). Hence both modifications contain about the same amount (about 0.4 mg.)

* N indicates "neutral," P "protamine," and H "Hagedorn," in whose laboratory *isophane or NPH insulin was developed.*

protamine. NPH insulin contains less zinc. Their speed, intensity, and duration of action and fields of usefulness are similar (see Fig. 34-1).

Lente insulin is a suspension of insulin with zinc in an acetate buffer. In contrast with other depot preparations it contains no protein precipitant. It was developed by Hallas-Møller and colleagues in Copenhagen in 1952 as a mixture of 3 parts amorphous zinc insulin crystals with prompt action ("semilente") with 7 parts crystalline zinc insulin with slow action ("ultralente") (8). Figure 34-2 gives comparative time action curves with these preparations. As marketed in the proportions indicated it fits the needs of most diabetic patients. Its timing of action is similar to that of the other intermediate preparations (Fig. 34-1).

*Severe and labile** *forms* of diabetes are best treated with the intermediate-acting insulins. Their appropriateness is suggested by any or all of the following behavior characteristics of individual cases:

High insulin requirement (more than 30 units daily), as a rule.

Almost all childhood and juvenile diabetes, except early in its course in some cases.

Many adult cases, particularly (1) those who are thin, and (2) after long duration of the disease.

Instability of balance between food and insulin.

Insulin shock during the night with long-acting insulins.

Glycosuria after meals, particularly in the afternoon and evening, with long-acting insulins.

Irregular, unpredictable behavior of glycemia and glycosuria on one dose daily of any depot insulin (*see below*).

In severe diabetes there are two techniques for using intermediate insulins routinely.

1. In those individuals who respond *consistently and predictably* (without unexpected episodes of heavy glycosuria or hypoglycemia) one of the intermediate insulins should be injected once daily before breakfast in a substantial dose, ordinarily 20 to 60 units for adults and adolescents. Food should be kept constant day by day. Breakfast usually is the smallest meal, lunch moderate, and dinner the largest. Because of peak insulin action 8 hours or so after injection it is usually wise to insert an afternoon snack. A bedtime feeding is optional because there is somewhat less danger of hypoglycemia during the night than with slow-acting insulins.

Dosage of intermediate insulin given in the morning is best judged by the late afternoon behavior, but it is also essential that fasting blood glucose values should not be unduly elevated nor depressed. The time

* Also known variously as "brittle," "unstable," "total," "growth-onset."

action of most intermediate insulins is such that the response in relatively stable diabetes will ordinarily be roughly equal at those two times of day. If it is not, the timing of the insulin, the food supply, or both require adjustment.

Most of the intermediate insulins can be accelerated and shortened in effect by adding unmodified insulin in small amounts. For lente insulin the supplement should be semilente; for mixtures and NPH insulin, solution of zinc insulin crystals should be used. Supplements should not exceed about 20 per cent of the insulin to which they are added, and if the supplement is added to the ampoule the modified insulin should be used within two or three weeks. The action of globin insulin is not accelerated much until about an equal amount of unmodified insulin is added to it (12). The same is true for protamine zinc insulin (3). It should be recalled, however, that the timing of action of these two preparations, and consequently their modifications, is quite different. The former is relatively fast and not prolonged and the latter slow and sustained.

2. Because *labile diabetes* is the most severe of all human forms of the disorder, it is the most difficult to manage smoothly. Waves of heavy glycosuria (sometimes with ketonuria) or of hypoglycemia with insulin shock appear unpredictably and without evident cause. Severe acidosis occurs with ease. Trauma, infection, anesthesia, and other acute complications are dangerous because they may lead to sharp increases in insulin requirement or acidosis. Routine insulin dosage is often large, but not necessarily so because many such patients are children, or are very sensitive to insulin, thus needing relatively smaller amounts.

It is estimated that some 10 per cent to 20 per cent of all patients using insulin have diabetes of this severity. It is characteristic that they cannot be kept "sugar-free" without great danger of severe insulin shock or other intolerable penalties of treatment. On that account, all authorities accept and condone some glycosuria as the lesser of two evils. The accepted doctrine approves of as little glycosuria as compatible with relative freedom from insulin shock and absence of ketosis In most instances glycosuria need not exceed about 25 Gm daily.

Allowances of carbohydrate should be somewhat more generous in diabetes of this type than when it is milder. There are several reasons for this. Glucose lost in the urine should be replaced. Postprandial glycosuria is often unavoidable. Extra carbohydrate feedings are desirable between meals to forestall reactions, especially during exercise. Children and young adults need more antiketogenic protection than old people.

Unmodified insulin in frequent dosage is undoubtedly the most pre-

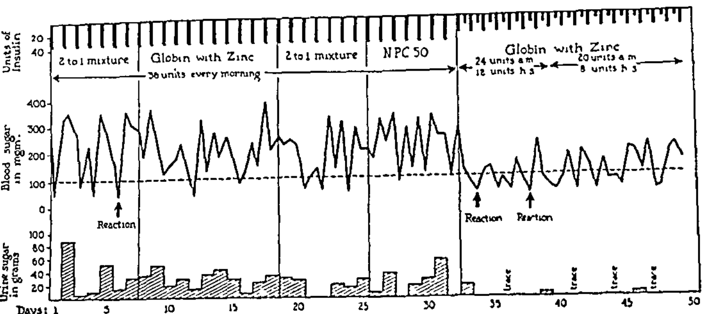


FIG. 34-3. Irregular behavior of labile diabetes on single daily doses of 3 intermediate insulins (first 32 days) contrasted with better control when one of the insulins was given twice daily instead of once (last 18 days). Diet constant throughout.

dictable insulin for treatment of diabetes of this severity. An injection every 6 hours or so, with food, including an injection at midnight or later during normal sleeping hours, will provide the best possible control with the least danger of reactions. But the inconvenience of a frequent injection routine such as this makes it virtually intolerable. It was the chief cause of the need for and development of the depot insulins.

A good compromise between accuracy and convenience in the treatment of diabetes as severe as this is the use of a fairly quick-acting insulin modification twice daily. Any of the following is suitable: a mixture of three or four parts of regular insulin to one part of protamine zinc insulin; globin or NPH insulin with regular insulin added to speed and intensify effect and increase predictability. From two thirds to three fourths of the insulin needed daily is given before breakfast and the balance at bedtime, along with a lunch. Interval feedings are given in the mid-morning and mid-afternoon; hence, regular meals are somewhat smaller, and glycosuria following them likely to be less in amount. The size of the morning dose is judged by the behavior in the late afternoon and evening; the night dose by that before and after breakfast.

Figure 34-3 illustrates the improvement in control accomplished by the simple substitution of this technique for the conventional one daily dose in a case of labile diabetes. Responses such as this are common.

Unmodified Insulin: Acute Complications

In modern diabetes therapy there are only two indications for the use of unmodified solutions of insulin.*

1. To accelerate and intensify the action of depot insulins. This has been discussed in the preceding section on intermediate insulins.

2. To adjust insulin dosage rapidly to suddenly increased demands during acute complications in diabetes.

Acute illnesses, which most commonly demand vigorous use of prompt-acting insulin solutions promptly, are acidosis, acute infection, surgery, anesthesia, and trauma. All except severe grades of acidosis can be managed easily by a simple and reliable method of using ordinary insulin. Precomatose and comatose states require more aggressive methods (see Chap. 37).

When heavy glycosuria, with or without ketonuria, exists as a result of acute illness it can be eliminated within 24 hours, as a rule, with ordinary insulin injected at 6-hour intervals (see Fig. 34-4).

* *Even though semilente insulin is a suspension,* its action is so fast that its indications are almost the same as for those of insulin solutions, except that when used as a supplement it should be added only to lente or ultralente insulin.

Every 6 hours the patient receives about 40 Gm. of carbohydrate by mouth or by injection, depending upon the alimentary capacity. This should be given at night as well as by day. Uniformity of sugar supply eliminates food from consideration as a possible variable affecting glycosuria.

Every 6 hours a dose of ordinary insulin is injected. Its size depends upon the intensity of the complicating illness, the inherent severity of

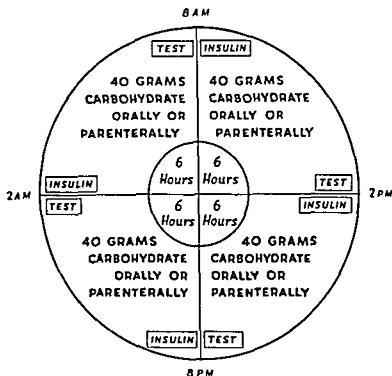


FIG. 34-4. Emergency management of diabetes during acute complicating illness by injection of unmodified insulin at 6-hour intervals night and day. Carbohydrate supply must be kept constant. Six-hour urine sugar tests determine subsequent 6-hour insulin dosage.

the diabetes, and the degree of insulin sensitivity. Except in severe acidosis, 10 to 40 units every 6 hours usually will suffice.

The size of each insulin dose is judged according to the observed effects of previous amounts A simple urine test (preferably on a short collection near the end of each 6-hour period) demonstrates the effect of insulin given previously. As long as heavy glycosuria exists, dosage should be increased. Reduction in glycosuria calls for caution, but not omission nor necessarily even reduction of the next 6-hour dose.

Insulin reactions or subnormal blood sugar values demand reduction but not omission of the next 6-hour dose. A constant 6-hour dosage can be established within 48 hours, as a rule. It should neither permit much glycosuria nor cause hypoglycemia when given repeatedly. On improvement in the general condition, the total 24-hour dosage can be replaced with a single large dose of one of the depot insulins and a diet of three meals served at customary intervals.

INSULIN SENSITIVITY* AND RESISTANCE

There are marked differences in individual response to given amounts of insulin. They can be demonstrated by standard "insulin tolerance" tests (Chap. 30), and by the contrasting insulin needs of various individuals and of the same individual under various circumstances. Many of the reasons for these differences are known; some are not.

Endocrine

In general, anterior pituitary, thyroid, or adrenocortical and medullary hypofunction causes increased sensitivity to insulin and hyperfunction causes resistance (Chaps. 15–18). The α-cells of the islets of Langerhans may act similarly. The responsible mechanisms involve increased production of glucose from protein with the adrenocortical hormones and from glycogen with epinephrine, vasopressin, and glucagon.

These relationships are of fundamental importance in the diagnosis of endocrine abnormalities, both in the presence and in the absence of diabetes, in the therapeutic use of those hormones, and in therapy with *insulin when endocrinopathies or hormone therapy cause or complicate* diabetes mellitus.

Obvious implications as to *treatment* are (1) correction of the endocrinopathy if possible; and (2) adjustment of insulin dosages accordingly.

Infection

Even mild infection in diabetes is likely to cause hyperglycemia, glycosuria, and acidosis, with abruptly increasing insulin requirements. The same is true for trauma, surgery, and general anesthesia. If not

* Although the term "sensitivity to insulin" sometimes is used to mean allergic reaction (usually urticarial) to insulin by sensitive individuals, it is not the frame of reference in this section. The terms "sensitivity" and "resistance" or "insensitivity" as used here refer to the magnitude of the physiologic response to standard amounts of the hormone.

compensated promptly a vicious circle is established, which may end fatally. Although under certain circumstances gamma-globulins act as antibodies, that does not appear to be the mechanism for the increased insulin need in infection and analogous states. The known factors of stress, increased protein metabolism, increased total caloric production, and acidosis are more logical causes.

Treatment consists of insulin and glucose in sufficient amounts to correct hyperglycemia, glycosuria, and acidosis until the acute complication subsides or is eliminated by appropriate means (*see section on unmodified insulin and acute complications above*).

Acidosis

Diabetic patients in advanced stages of acidosis need much larger amounts of insulin than in its absence. Hundreds and even thousands of units daily are required.*

An insulin antagonist, demonstrated by Field and Stetten in the serum α-globulin fraction in severe acidosis, appears to be responsible for this type of resistance. It and the insensitivity to insulin disappear within a few hours on treatment, along with the acidosis. Its origin is unknown.

Treatment of this form of insulin resistance is the same as that for diabetic acidosis.

Allergy

Although insulin resistance may coexist with local or general urticarial or other reaginic manifestations in diabetes, more often the two phenomena occur independently. They are different entities with different mechanisms and treatments (Chap. 20).

Insulin resistance of an allergic character has been seen clinically in hepatic cirrhosis, in hemochromatosis, in hematologic disorders, and in the absence of any known cause.

Although insulin is a weak antigen, it causes production of immune serum when given to animals of a species different from that of its origin. Minor differences in amino acid linkage have been shown in insulins from various animals. Using iodine-tagged insulin as a tracer, Berson, *et al.*, have demonstrated insulin-binding globulin in the plasma of diabetic patients after continued use of conventional insulins. Such antibodies are specific for the species of insulin used previously. Ordinarily they exist in amounts inadequate to cause clinical insulin insensi-

* A diabetic in coma is not necessarily in diabetic coma. Large amounts of insulin are dangerous and will not correct unconsciousness due, for example, to cerebral hemorrhage, trauma, barbiturates, or insulin shock.

tivity, but under certain conditions, many of them unidentified, an anamnestic cycle is created by the heterologous insulin. Globulin production is increased measurably, marked resistance appears, and massive, progressively increasing amounts of insulin are required for control of the diabetes (Chap. 20) (4).

Until this form of insulin resistance has been identified in a given individual, as with other types it is treated by giving increasing amounts of standard insulins. It may be suspected when there are progressively increasing insulin needs without obvious cause. It can be proved by finding abnormal increases in serum total and gamma-globulin fractions that inhibit the activity of the insulin used when mixed with it and assayed by appropriate biological methods.*

Theoretically all that is then necessary is to substitute insulin made from the pancreas of a different species. Too often that is not readily available, however, and even if it were it would probably lead also to its own antibody production in a susceptible individual unless it were human insulin. No instance of such a clearly effective substitution has been reported.

Corticotropin and cortisone in ordinary amounts have been dramatically effective in several cases of this type of insulin resistance (4). Insulin requirements fall abruptly and abnormal serum globulin values more slowly. There is grave danger from hypoglycemia while massive doses of insulin thus are being reduced, and large amounts of glucose may be required for a few days. The duration of the reduced insensitivity has not been documented. It may last for weeks or months.

SKIN REACTIONS TO INSULIN

Common skin lesions following the use of insulin are infection, urticaria, and hypertrophy and atrophy of subcutaneous fat.

Infection

Sterile techniques, as required for parenteral injection of any substance, are particularly important with insulin because it is injected every day, usually by laymen. Necrosis, inflammation, or abscess formation may appear at the site of an injection.

The best *treatment* is preventive, consisting of adequate training and daily care. If infection appears it is managed by conventional methods, usually with increased amounts of insulin temporarily.

* Injection of single doses of the test and control mixtures into rabbits, rats, or mice and judging insulin responses by comparing consequent reduction in blood glucose (rabbits or rats) or appearance of convulsions (rats or mice).

Urticaria

Generalized severe urticaria resulting from insulin sometimes appears in sensitive individuals. It may respond to antihistaminics, to the use of insulin from another species, to steroid therapy, or to desensitization by standard techniques with the insulin used.

Localized wheals frequently appear at the site of injection, particularly with depot insulins, in the thighs, and in women at or after menopausal age. Usually they are not serious, disappearing within a few hours. They are often prevented by injection of the insulin *underneath* instead of *into* the subcutaneous fat. Substitution of another form of insulin or use of antihistaminics may be effective.

Lipodystrophy

Either hypertrophy or atrophy of subcutaneous fat may occur at the site of insulin injection.

Fibrous, *fatty tumors* are caused by habitual injection into the same area. Children are likely to cause them because such an area becomes relatively painless on insertion of the needle. The practice is objectionable, however, for cosmetic reasons and because irregular insulin responses can be expected from such an avascular, scarred, and hypertrophied depository.

Lipomas can be prevented by systematic rotation of injection sites. They get smaller when abandoned but may not disappear.

Localized *atrophy* of subcutaneous fat is a curious consequence of subcutaneous use of insulin. The panniculus vanishes completely at the injection site, leaving disfiguring depressions several inches in diameter, commonly in the upper arms or legs. Atrophy occurs largely in women and children, usually after continuous injection into a given site, but at times after only a few injections. It is not common, but its cause is unknown and when it appears it usually is permanent.

The only known therapeutic measures are (1) meticulous care not to repeat injection into the same location more often than every few weeks, using a diagram if necessary to insure that this is done, and (2) use of the abdomen, buttocks, and back for injection.

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Chapter 35

ORAL DRUGS IN DIABETES

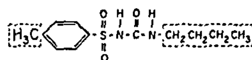
Robert H. Williams

For many decades oral drug therapy for diabetes has been sought and, as reviewed by Lewis and others, a large number of compounds have been tested. Significant progress, however, has been made only in the last few years, chiefly with sulfonamides. A summary of the most important observations with these and some other hypoglycemic compounds is presented in this chapter.

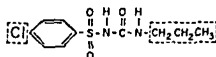
SULFONYLUREAS

In 1942, while studying the antibacterial effects of 5-i-propyl-2-p-aminobenzenesulfonylamino-1,3,4-thiadiazole in typhoid fever, Janbon observed that this compound caused hypoglycemia with its characteristic symptoms and signs. Thereafter, Loubatieres and others conducted classic investigations on the mechanism of production of the hypoglycemia and concluded that the principal action was stimulation of the pancreatic β-cells to increased insulin secretion. Many of the details of these early studies have been reviewed recently by Loubatieres and others and are not discussed here. Although other thiadiazoles were also shown to produce hypoglycemia, no reports of clinical application

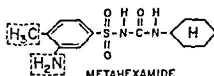
appeared until after the accidental discovery of the hypoglycemic action of another type of sulfonamide, carbutamide, a sulfonylurea, the antibacterial effect of which was being studied. Carbutamide is the generic name for 1-butyl-3-*p*-aminobenzenesulfonylurea. This compound has been investigated in the laboratory very extensively and has been used in treating several hundred thousand diabetics. However, another closely allied drug, tolbutamide, soon proved to be preferable because it produced fewer side effects. Tolbutamide (Orinase, Rastinon, Dolipol, Artosin) is the generic name for 1-butyl-3-*p*-tolylsulfonylurea (Fig.



TOLBUTAMIDE



CHLORPROPAMIDE



METAHEXAMIDE

Fig. 35-1. Sulfonylureas with marked hypoglycemic action. The sulfonamide radical (SO_2NH_2) is the active part of the molecule.

35-1). Whereas tolbutamide has a methyl radical in the para position, carbutamide has an amino group, but otherwise the structures are identical. Thus carbutamide is a sulfanilamide while tolbutamide is not, only the former is bacteriostatic. Tolbutamide has been valuable in the treatment of a large number of diabetics and has manifested relatively few and mild side effects.

Many other sulfonamides have been demonstrated to produce hypoglycemia. The sulfonamide group is necessary for the hypoglycemic action, but slight modifications in the side chains have been shown to influence markedly the hypoglycemic capacity and the toxic manifestations. There are two compounds in this group that are much more potent than tolbutamide (Fig. 35-1). Both of these are sulfonylureas:

chlorpropamide (1-propyl-3-*p*-chlorobenzenesulfonylurea) and metahexamide (1-cyclohexyl-3-[*m*-amino-*p*-methylbenzenesulfonylurea]). A summary of some detailed investigations with tolbutamide follows, along with brief comparisons of certain effects of chlorpropamide (*Diabinese*) and metahexamide.

Metabolism

Tolbutamide is an odorless white crystalline compound which is poorly soluble in water but rapidly absorbed from the stomach and

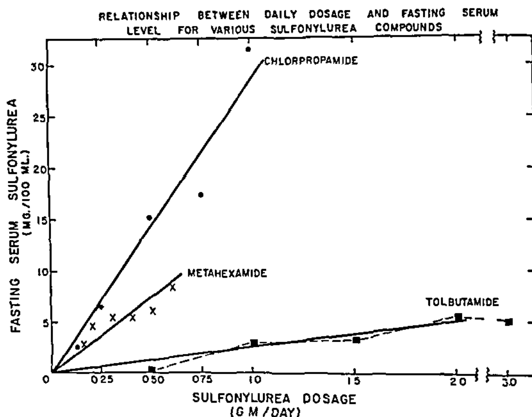


FIG. 35-2. Mean blood level with various daily doses of sulfonylureas. With a given dose the highest level is obtained with chlorpropamide and a somewhat lower level with metahexamide. Several times as much tolbutamide must be given to obtain drug levels comparable with those of chlorpropamide or metahexamide (Data kindly supplied by Dr. Stefan S. Fajans.)

duodenum. Some of the drug is demonstrable in the blood within 30 minutes and a peak concentration is reached in 3 to 5 hours. It is bound to plasma albumin and is stated to remain in extracellular spaces. Its major excretory product, butyl-*p*-carboxy-phenylsulfonylurea, is ex-

creted rapidly in the urine. The latter results merely from the oxidation of the methyl to a carboxyl group and has no hypoglycemic action; it is not prone to produce crystalluria since it is soluble to the extent of 1 gm./1 ml. of water. Tolbutamide is not acetylated. Its half-life is about 4 to 5 hours.

Chlorpropamide is an odorless white crystalline powder insoluble in water but soluble in alcohol or chloroform. It is not bacteriostatic. It is absorbed rapidly from the gastrointestinal tract, being detectable in the blood in 1 hour and attaining a maximal concentration, from a single dose, in 2 to 4 hours. It is bound to plasma protein and is partially excreted very slowly in the urine in unchanged form. It has not been reported to produce crystalluria. After a single dose to man the half-time disappearance rate is 35 to 40 hours or almost 10 times slower than that of tolbutamide. Within 96 hours 80 to 90 per cent of a single dose is excreted in the urine. With daily doses of 250 to 500 mg. a plateau of the blood concentration is reached in 3 to 5 days. After therapy for 16 days, 20 additional days are required for clearance of the drug from the blood. Chlorpropamide, as well as tolbutamide, accentuates the sedative effect of barbiturates.

It takes several times as much tolbutamide to produce the same drug blood level as it does of chlorpropamide or metahexamide (Fig. 35-2); moreover, chlorpropamide accumulates in the body more than does metahexamide.

As discussed later, all three drugs are highly toxic in doses many times those indicated clinically, but acute toxic effects at the clinical level are very few and chiefly mild.

Hypoglycemic Actions and Their Mechanisms

In the studies with the thiadiazole sulfonamides and with the sulfonylureas, it soon became apparent that β-cell function of the pancreatic islets was necessary for hypoglycemic action It was also demonstrated that the pattern of the hypoglycemic response was not the same in diabetics and nondiabetics and that euglycemia was attained later with the sulfonamides than with a comparable hypoglycemic dose of insulin (Fig. 35-3).

An enormous number of studies has been directed toward establishing the mechanism of action of these compounds, but many conflicting reports have appeared. Much of the confusion has resulted from inadequate consideration of such factors as dosage of insulin versus that of sulfonylureas, routes and rates of administration, species variations, and preparation of the in vitro and/or in vivo specimen Although complete proof is lacking, there is a considerable quantity of informa-

tion suggesting that the *chief action of sulfonylureas is to stimulate increased production of insulin* with effects elsewhere of such a mild degree as to be essentially unapparent unless supplemented by increased insulin action. The most pertinent of the supporting data are now discussed, with emphasis on the positive results that suggest the aforementioned mechanisms of action.

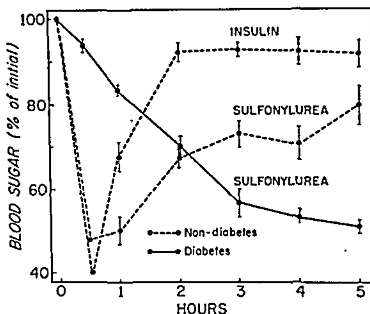


FIG. 35-3. When tolbutamide was given orally to fasting nondiabetic subjects, 50 mg./kg, a rapid drop in the blood sugar occurred, comparable to that following insulin administered intravenously, 0.1 unit/kg. However, after tolbutamide, there was a much slower return to normal. The fall in blood sugar produced by sulfonylurea in subjects with diabetes was much slower than in those without this disease. (Replotted from charts by Mirsky, by permission Filed at Library of Congress, Catalog Card No. 56-13327)

PANCREAS. There is essentially complete agreement among investigators that without exogenous insulin, active β-cell function is necessary for hypoglycemic response to the sulfonylureas. Totally depancreatized animals or patients and animals with severe alloxan diabetes fail to respond. No consistent significant changes have been demonstrated in the α- or Δ-cells nor in the quantity of glucagon assayed in the pancreas or plasma. However, anatomical changes do occur in the β-cells, along with a decrease in the insulin content of the pancreas, there also are a few reports of increased insulin content of the plasma.

Within a few hours of the initiation of tolbutamide administration to animals there is apparent marked degranulation of the β-cells and an increase in nuclear volume. The islet weight increases but the total pancreas weight decreases. With continuation of therapy for many days these pancreatic changes tend to regress.

Doses of tolbutamide too small to produce hypoglycemia when injected into the femoral vein or portal vein cause marked hypoglycemia when infused into a pancreatic artery. Moreover, in cross-circulation experiments the recipient animal, whether normal or diabetic, develops hypoglycemia when he receives his blood directly from a pancreatic vein of a tolbutamide-treated intact animal, but not when the blood is received from a mesenteric vein or femoral vein. Apparently the relatively high concentration of the drug circulating through the pancreas stimulates secretion of insulin by the β-cells.

INSULIN DEGRADATION. In relatively large doses the sulfonylureas inhibit insulin degradation by liver homogenate. If this effect were produced in vivo, the effectiveness of a given quantity of insulin would be increased. Thus, the endogenous supply of insulin could be extended to a satisfactory level in some diabetics. Ordinarily, however, with tolbutamide this action apparently occurs to a slight degree only, or not at all, because the dosage used clinically is apparently inadequate for this purpose. Moreover, tolbutamide has been reported to remain extracellular whereas the enzymatic degradation of insulin occurs intracellularly. If such were the case, however, difficulty would arise in explaining some of its other known actions.

DIRECT AND INDIRECT EFFECT ON MUSCLE, ADIPOSE TISSUE, AND LIVER. If tolbutamide increases insulin secretion, as suggested by the previous discussion, it should be possible to demonstrate insulin-like actions in certain tissues of intact animals treated with this compound. This subject is now considered along with direct actions of tolbutamide in tissue.

Muscle. Numerous in vitro studies have failed to reveal any direct effect of tolbutamide upon the glucose uptake by muscle, an action readily demonstrated with insulin. In the occasional instance when an increased glucose uptake has been stimulated by sulfonylurea in vitro, the effect might be attributable to decreased insulin degradation or to inhibition of cellular oxidation; cellular anoxia of varying causes frequently increases glucose uptake by a compensatory mechanism. Chlorpropamide has been demonstrated to cause increased glucose uptake in vitro as well as in vivo; it also has been found slightly to inhibit oxygen consumption

Tolbutamide administered to animals eviscerated, depancreatized, or severely alloxanized has not increased glucose utilization. However, in

the presence of actively functioning β-cells it has been shown to increase glucose oxidation and, like insulin, to increase A-V glucose difference and to lower the threshold for transfer of glucose into cells.

Adipose Tissue. Relatively few studies dealing with the effect of sulfonylureas on adipose tissue have been reported, but fed rats receiving this therapy have been found to exhibit increased glycogen deposition by interscapular fat pads; this was not found to be the case with fasted rats.

Liver. Since the actions of insulin in the liver have not been appropriately elucidated, it is not surprising that interpretations of the actions of the sulfonylureas have been difficult and confusing. Some of the tolbutamide actions upon the liver apparently are direct while others may result from increased insulin secretion; in some instances it is difficult to differentiate between these. Without considering, at present, the conditions under which the observations were made, the following effects of tolbutamide may be listed: decreased hepatic glucogenesis, decreased glucose-6-phosphatase activity, decreased glycogenolysis, decreased glucogenic response to epinephrine and glucagon, decreased rate of conversion of fructose and galactose to glucose, increased hepatic vein pyruvate, decreased phosphorylase reactivation by liver homogenate, inhibition of alanine transaminase, inhibition of acetaldehyde dehydrogenase, inhibition of acetylation of p-nitroaniline, and increased serum glutamic oxaloacetic transaminase. Some of the aforementioned results may be altered, even to an opposite direction, with changes in the dose of tolbutamide, preparation of the animal, etc. Thus numerous factors must be considered in analyzing the results and in this light the following discussion of the net effects of tolbutamide is presented.

DISCUSSION OF NET ACTIONS OF TOLBUTAMIDE. For the reasons presented previously, the major action of tolbutamide seems to be stimulation of increased secretion of insulin from the β-cells; in the absence of these cells tolbutamide produces little or no hypoglycemia. The increase in insulin secretion is apparently only of slight magnitude but extends over many hours. The insulin secreted goes first to the liver and, consequently, it has the first opportunity to act on the liver and also to be degraded by that organ. Tolbutamide, like very small doses of insulin infused into the portal vein or injected subcutaneously, has been shown to cause hypoglycemia and to decrease hepatic glucogenesis without affecting the peripheral utilization of glucose; larger doses of insulin increase glucose utilization and under certain conditions tolbutamide has been shown to promote the same. Since tolbutamide does not evoke either response in depancreatized animals and since it produces com-

parable hypoglycemia in hepatectomized and in intact animals, the major changes in the liver seem to result directly or indirectly from increased insulin secretion. However, a direct action of tolbutamide in the liver must supplement the hepatic hypoglucogenesis caused by insulin, because there has been produced in depancreatized but not in hepatectomized animals more hypoglycemia with tolbutamide plus insulin than with insulin alone. Tolbutamide has been reported to decrease hepatic glucogenesis in depancreatized animals. The direct hepatic effect of tolbutamide seems to be too weak to produce hypoglycemia without the permissive role of insulin. The mechanism by which insulin or tolbutamide decreases hepatic glucogenesis is incompletely understood. Each decreases gluconeogenesis—tolbutamide relatively more than insulin. With each there is a decrease in glucose-6-phosphatase, but this seems to relate to insulin action since tolbutamide does not manifest this effect in alloxanized animals. Tolbutamide produces a higher liver glycogen concentration, possibly via inhibition of phosphorylase activity.

Tolbutamide increases glucose utilization by peripheral tissues but only to a degree comparable to a small dose of insulin. This apparently results from the very slight increase in plasma insulin produced by tolbutamide. Since, following tolbutamide administration, many biochemical alterations have been of different degree from those observed with insulin, numerous investigators have concluded that tolbutamide did not act chiefly by increasing insulin secretion and/or action. Among the problems have been the observations that insulin and tolbutamide often have not produced comparable changes in plasma pyruvate, lactate, α-ketoglutarate, phosphorus, potassium, amino nitrogen, and lactic dehydrogenase. However, in order to make the most appropriate comparisons, the administration of insulin should simulate closely the supposed pattern of increased insulin stimulated by tolbutamide, namely, a slightly increased plasma concentration for several hours with the insulin passing first through the liver. The few studies conducted in this manner have, in fact, yielded many similarities.

A few additional differences between tolbutamide and insulin action are as follows: hypophysectomized animals have a hypoglycemic response to insulin as great as do adrenalectomized, but adrenalectomized animals respond much more to tolbutamide than do hypophysectomized. Indeed, adrenal demedullation alone greatly increases the tolbutamide response. For reasons unknown, tolbutamide helps prevent the diabetogenic action of somatotropin and it does not cause β-cell enlargement in hypophysectomized animals.

Some of the patterns of blood sugar responses and factors contribut-

ing to them are discussed later under the heading, "Acute Screening Test."

Compared with the number of observations made with tolbutamide and carbutamide, relatively few have been made on the mechanism of action of chlorpropamide and metahexamide, but available information indicates that their actions may be similar to those of tolbutamide.

Clinical Responses with Tolbutamide

Early investigations with both carbutamide and tolbutamide demonstrated that certain clinical characteristics tended to favor hypoglycemic responses. Among these were old age, recent onset of diabetes, small insulin requirement, and other features influencing the type of diabetes. It seems probable that the extent to which each of these factors influences the responsiveness of diabetics to sulfonylurea therapy depends upon the degree to which they affect insulin secretion and effectiveness. Some of these factors are now considered individually, drawing not only upon our own experiences in the treatment of approximately 200 patients, but also particularly on the survey by O'Donovan of co-operative studies of 9,168 subjects conducted by 420 physicians and on those of Mehnert and associates in 1,030 diabetics. In these, as in essentially all other reports, the patients investigated were largely selected from groups anticipated to yield favorable responses.

There is great variation among investigators in the criteria selected for characterizing different degrees of response to therapy. Those of Mehnert, *et al.*, are presented in Table 35-1; none of the responses was designated as "excellent" by that group. In the co-operative study the designations consisted of "excellent," "good," "fair," "poor," or "failure." Although the criteria associated with the designations varied, they were of advantage in evaluating the influence of different factors on drug responsiveness.

EFFECT OF DOSAGE. There have not been reports of careful evaluation of dosage. A common dosage pattern has consisted of the administration of 3 gm. the first day, 2 gm. the second, and 1 gm. daily thereafter, with adjustments upward or downward in accordance with the response of the patient. Approximately two thirds of the patients receive a maintenance dose of 0.5 to 1.5 gm. daily, 90 per cent receive 2 gm. or less daily Rarely is it advantageous to administer more than 1.5 gm. daily and in all patients the minimal dose producing optimal effect should be sought. In most instances the compound is administered once daily, preferably before breakfast. Because the half-life of the compound is only a few hours, it is desirable to administer it in two doses daily, before breakfast and supper, when large doses are required. When the

drug induces gastrointestinal symptoms, some relief is afforded by administering it with or immediately after food.

EFFECT OF AGE. O'Donovan reported that a good or excellent response was obtained in 65 to 69 per cent of those whose disease presented after the age of 40 years and in at least 40 per cent of those between 20 and 39.9 years. As shown in Figure 35-4, the older the subject at the onset of diabetes and at the initiation of tolbutamide therapy the higher is the incidence of favorable responses. The age of the patient at the time that his diabetes becomes manifest influences the responsiveness

TABLE 35-1. BLOOD SUGAR LEVELS AND AMOUNTS OF SUGAR IN URINE USED AS STANDARDS OF CONTROL*†

	Degree of control‡			
	Good		*Fair*	
Relation to food	*Blood sugar mg./100 cc.§*	*Urine sugar per cent*	*Blood sugar mg./100 cc §*	*Urine sugar per cent*
Fasting	110	Trace	130	0 1
1 hr p c.	150	0 3	180	0 5
2 hr. p c.	130	0 1	150	0 3
2 hr. p.c.	110	Trace	130	0 1
Urine sugar in 24 hr.	2 Gm. or less		5 Gm. or less	

* By permission, after Mehnert, H , Camerini-Davalos, R , and Marble, A : *J A M A 167* 818, 1958

† For classification as to degree of control, the majority of values must conform with the standards listed in the table All others are considered poor

‡ These standard values are the highest acceptable

§ "True" glucose as determined by the Somogyi-Nelson procedure

to tolbutamide more than does the age at which this therapy is initiated. The basic nature of the disease is more important than the age of the patient per se. In general, however, the older the patient at the onset of diabetes the milder is the disorder and the more responsive to sulfonylureas. Females, particularly between 20 and 40 years of age, respond much better than males in this age range.

EFFECT OF DURATION OF DIABETES. The percentage of tolbutamide failures increases with increase in the duration of diabetes preceding the administration of tolbutamide This is of much less consequence, however, in patients with maturity-onset diabetes than in those with juvenile diabetes.

CORRELATION WITH PRIOR INSULIN THERAPY. As shown in Figure 35-5,

the larger the daily dosage of insulin and the longer this therapy before tolbutamide administration, the lower was the incidence of good or excellent responses to the oral drug. In reviewing the co-operative investigations, O'Donovan found that 53 per cent of 5,233 patients had previously shown an "excellent" or "good" response to insulin, while 59 per cent of the insulin-treated group showed an "excellent" or "good"

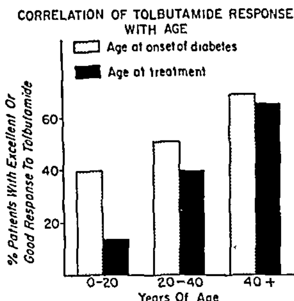


FIG. 35-4. With increase in age at onset of diabetes and also in age at treatment there was a corresponding increase in effectiveness of tolbutamide therapy. Apparently patients developing diabetes at older ages produce a more normal supply of insulin with or without tolbutamide treatment (Plotted from data kindly supplied by Dr. C J. O'Donovan.)

response to tolbutamide (Fig. 35-5). With tolbutamide there was "highly successful control of glycemia and glycosuria" in 79 per cent of those who had previously been treated with 10 units or less, in 57 per cent with 20 to 30 units, and in 36 per cent with 41 to 50 units.

CORRELATION WITH PREVIOUS DIET THERAPY. Of 2,159 diabetics, only 19 per cent achieved "excellent" or "good" control when previously treated with diet alone, but 75 per cent of this group attained "excellent" or "good" control with tolbutamide plus diet (Fig. 35-6). Whereas the statement is often made that tolbutamide exerts its best effect in the group that should respond well to diet restriction, ex-

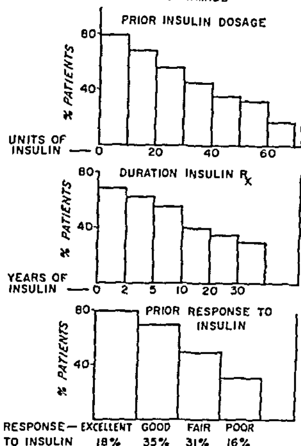


Fig 35-5. The smaller the dosage of insulin required preceding tolbutamide therapy and the shorter the course of insulin, the higher was the incidence of favorable response to tolbutamide. From the lower chart it is seen that in this group of patients previously treated with insulin a better response is obtained with tolbutamide than with insulin (Data supplied through the courtesy of Dr. C. J O'Donovan)

perience has demonstrated that many patients treated with diet alone do not adhere sufficiently to the prescribed diet in spite of repeated insistence by their physician Since the diabetes of many of these patients is satisfactorily controlled with sulfonylurea therapy even though they continued to diet improperly, it seems that the advantages of the drug outweigh the disadvantages. However, diet therapy should be utilized as much as possible, both with and without drugs.

CORRELATION WITH BODYWEIGHT. The degree of obesity per se has been regarded by many investigators as playing no role in the responsiveness to tolbutamide, but in a few studies obese subjects have responded better than non-obese. The response is distinctly better when there is simultaneous reduction of the obesity by diet restriction.

EFFECT OF TYPE OF DIABETES. Although much is unknown about the pathogenesis of the commoner types of diabetes, the information available suggests that the chief factor in determining the response to tolbutamide is the amount of increase in insulin secretion that can be stimulated. All the previously discussed factors seem to be of less prime

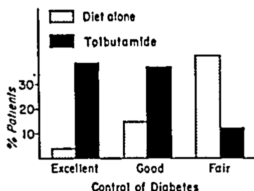


FIG. 35-6. A far better response is obtained with tolbutamide plus diet than with only the latter. (Data supplied through the courtesy of Dr. C. J. O'Donovan)

importance. Some of the observations are now analyzed in this light. Patients who can secrete no insulin, e.g., depancreatized subjects, have no hypoglycemic response to tolbutamide. Patients developing diabetes spontaneously before adulthood (juvenile diabetes) tend to have progressively less insulin in the pancreas and plasma with increased duration of their diabetes and require increasing quantities of exogenous insulin. As the diabetes becomes progressively more unstable it becomes less responsive to tolbutamide, until eventually very few juvenile diabetics respond. The older the subject when he develops diabetes the better supply of insulin he tends to have in the pancreas and plasma, the smaller the insulin requirements, and the more stable the diabetes. The intensity of the diabetes tends to increase but slightly in older diabetics and there continues to be good responsiveness to sulfonylureas.

Thus, the stable type of diabetes (maturity onset) responds much better than the unstable (juvenile). This concept is diagrammed in Figure 35-7, along with certain previously discussed observations related to the action of the sulfonylureas; not too much attention should be given to the exact height of the columns.

In general, poor responses to tolbutamide have been encountered in patients with diabetes resulting from pancreatectomy or extensive pancreatic destruction, hemochromatosis, and steroid diabetes. In the light

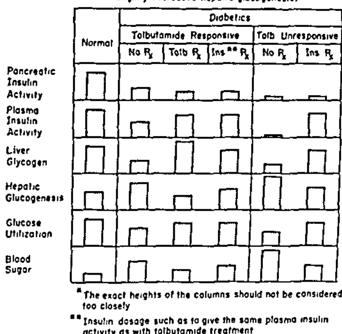


FIG. 35-7. An approximation of the action of tolbutamide

of the previous discussions it is evident that the extent of success with tolbutamide therapy depends very much upon the type of patients selected for therapy. In addition to considering the clinical features discussed, benefit is derived from an acute screening test now described

ACUTE SCREENING TEST. The administration of a large dose of tolbutamide to normal subjects apparently leads to the sudden secretion of insulin, manifested by a hypoglycemic response with a characteristic pattern (Fig. 35-3). The nadir is similar to that following insulin but euglycemia is attained more slowly, perhaps because of continued hy-

persecretion of insulin and decreased hepatic glucogenesis. The decrease in blood sugar in diabetics occurs more slowly, apparently owing to the slower discharge of insulin. The difference in the response of diabetics and nondiabetics is so great that Unger and others have used this principle in diagnosing diabetes. Twenty minutes following intravenous tolbutamide, the glucose level of 94 per cent of diabetic patients remained at 84 per cent or more of the pretest value, whereas in 96 per cent of nondiabetics it had fallen to 80 per cent or less of the pretest values.

Mehnert, *et al.*, in conducting more than 500 screen tests concluded that: (a) a successful outcome of tolbutamide therapy is more likely to occur the closer the fasting blood sugar is to normal, and (b) a "satisfactory" sulfonylurea test is one in which a single dose of the drug produces euglycemia within a few hours. For example, a drop from 130 to 80 more accurately signifies a successful therapeutic response than a drop from 300 to 150. The test is conducted as follows: (1) In stable diabetics, long-acting insulin is omitted for 2 days and is replaced either by short-acting insulin or none. (2) On the day of the test, insulin and food are withheld until completion of the test. (3) A fasting blood sugar determination is obtained. (4) Three gm. of tolbutamide is given orally. (5) Another blood sugar determination is obtained 4 hours later. The test is more helpful in designating those patients who will not have a response to long-term therapy than those who will. For example, of 74 patients who failed to respond satisfactorily to tolbutamide therapy, only 5 had a positive (satisfactory amount of blood sugar lowering action) screening test, but one fourth of the subjects who later achieved good control had an unsatisfactory response with the screening test. The test did not help in predicting "secondary failures," described later.

NET INCIDENCES OF SUCCESSFUL RESPONSES AND FAILURES. As discussed earlier, many factors influence the therapeutic response to tolbutamide. Patients showing the best response tend to be elderly, with stable diabetes of recent onset, requiring little or no insulin. A satisfactory response to a tolbutamide screening test increases the chances of satisfactory response to long-term therapy.

In all reports there has been a selection of cases and the results depend upon the extent to which this has occurred. The term "primary failure" has often been applied when the therapeutic response has been poor within the first 1 to 4 weeks. "Secondary failure" is said to occur when the patient has been under satisfactory control for several weeks and then fails to respond adequately.

Mehnert observed primary failure in 17.6 per cent of 772 patients. Of those followed satisfactorily from 1 to 20 months, "good" control was obtained in 52.6 per cent and "fair" control in 18.5 per cent. Thus a total of 71 per cent had a "satisfactory" response during long periods. A total of 5.2 per cent experienced secondary failure; in some the failure was due to lack of adherence to the diet and gain in weight.

O'Donovan reported that "primary failure" correlated well with previous insulin dosage. For example, only 7 per cent of patients who had received 10 units daily failed to experience benefit from tolbutamide. Failure occurred in 36 per cent of those who had received 30 to 40 units and in 66 per cent of those who had more than 60 units daily. Delayed failure occurred in 5.1 per cent of 9,168 patients but in 2.7 per cent was attributed to gross dietary negligence, intercurrent infection or other specific causes; only 2.4 per cent were said to be idiopathic failures.

SIDE EFFECTS. Many thousands of patients have been treated with tolbutamide in the United States alone, but the side effects have been infrequent and mild. O'Donovan's review of 9,168 cases indicated an incidence of 3.2 per cent, with withdrawal of the drug in 1.5 per cent, compared with 1.1 per cent incidence of side effects in Mehnert's series of 772 patients. O'Donovan reported that 22 subjects (0.24 per cent) had hematologic disorders; 19 of these had transient leucopenia (1800 to 3900 leucocytes per cubic millimeter). In 9 of these the leucocyte count returned to normal in spite of continued treatment. In no instance did agranulocytosis develop. In all instances it is important to differentiate agranulocytosis and leucopenia because the pathogenesis and significance differ. Routine serial leucocyte counts are not indicated with tolbutamide treatment.

Skin reactions were found in 1.1 per cent of the patients, but the drug was discontinued in only 0.52 per cent. Most often the skin rash was a mild erythema or urticaria. The most frequent side effects consisted of gastrointestinal symptoms: anorexia, epigastric discomfort, and diarrhea. These occurred in 1.4 per cent but the drug was discontinued in only 0.7 per cent. Careful extensive studies have shown no evidence of hepatic or renal damage (Zeffren).

The incidence of hypoglycemic reactions is unknown, but they have been rare and mild, except for one instance in a malnourished diabetic, 86 years of age, whose food intake had been poor for days; he was found to have pronounced hypoglycemia before death. Whereas deaths from various causes have occurred in patients who were receiving tolbutamide therapy, it is difficult to be sure of the drug's contribution The evidence suggests that such must be extremely rare.

Comparisons of Responses to Chlorpropamide, Metahexamide, and Tolbutamide

Although there have been fewer studies with chlorpropamide and metahexamide than with tolbutamide, it appears that the major mechanism of action is the same, but a more marked and prolonged hypoglycemic response is obtained from the former two, attributable partially, at least, to greater accumulations of these two compounds in the body. Each of the three compounds is lethal if given in large doses, but the indicated therapeutic dose is relatively so small that side effects are not common. Whereas metahexamide offered promise because of its effectiveness in controlling many diabetics, it has produced jaundice in approximately 1 per cent of patients. Consequently, its usage in the United States has been discontinued.

According to Dr. Domenic G. Iezzoni (Pfizer) who kindly provided me with results of his recent review of co-operative investigations with chlorpropamide, side effects have occurred in approximately 8 per cent of 5,000 patients; in approximately 3 per cent the drug was discontinued because of the side effects. Gastrointestinal disturbances have been found in 2 per cent. Jaundice has occurred in approximately 0.3 per cent of the subjects. Biopsy of the liver in a few such patients has demonstrated intracanalicular biliary stasis. Some jaundiced patients have subsequently received tolbutamide and thereafter chlorpropamide again (lower dosage than formerly) without any apparent difficulty. Two deaths with jaundice have occurred, but whether they were due to the drug is uncertain. Approximately 3 per cent of the patients treated with chlorpropamide have developed maculopapular or urticarial rashes; 3 subjects developed exfoliative dermatitis, which cleared with dermatologic care and cessation of the drug. Leucopenia has occurred in 0.6 per cent of the patients but there has been no agranulocytosis; the leucopenia disappeared in all instances even though therapy was continued in some subjects. A few of these patients had previously developed leucopenia while receiving tolbutamide. One person developed thrombocytopenic purpura while receiving chlorpropamide, but it disappeared promptly with cessation of this drug and the administration of corticosteroid therapy. Mild and transient neurologic disturbances have occurred in 1.5 per cent.

As discussed earlier, chlorpropamide remains unaltered in the body for much longer intervals than tolbutamide. In equal dosage it produces more pronounced hypoglycemia and for much longer intervals. In normal subjects it has been found to produce hypoglycemia within one hour reaching a maximum in 3 to 6 hours and persisting for 24 hours.

The blood concentration of chlorpropamide required to produce a given degree of lowering of the blood sugar is only slightly less than that of tolbutamide, but chlorpropamide's much greater tendency to accumulate in the body necessitates smaller doses. The initial daily dosage in adults is approximately 250 mg. (less in elderly patients) and the daily maintenance dose usually is between 250 and 500 mg., although occasionally it may be as low as 100 mg. or as high as 1,000 mg. Doses larger than the latter should be used only under exceptional circumstances; the incidence of gastrointestinal side effects is high with doses greater than 1,000 mg. Only one dose daily of chlorpropamide is indicated, usually at breakfast.

Experiences with several thousand diabetics have demonstrated a higher incidence of satisfactory blood and urine sugar responses to chlorpropamide than to tolbutamide. Of approximately 1,700 patients over 40 years of age, treatment with chlorpropamide produced a good or fair response in 86 per cent; failure occurred in 14 per cent. Good responses have been obtained with chlorpropamide in approximately 60 per cent of patients who had primary failure with tolbutamide and in 80 per cent of those who had secondary failure. About 0.1 per cent of the patients have had secondary failure to chlorpropamide. It is noteworthy that an occasional patient experiencing secondary failure with tolbutamide has responded to chlorpropamide and subsequently responded again to tolbutamide. A few patients with primary or secondary failure with chlorpropamide have responded satisfactorily to tolbutamide. This drug has been used advantageously in a few unstable (adult-type) diabetics; in these cases the insulin dosage was reduced and smoother control was obtained. Metahexamide causes the greatest hypoglycemia of any of the sulfonylurea drugs. It is effective in blood concentrations much less than those required for chlorpropamide and tolbutamide. Metahexamide has been claimed by various investigators to have a blood sugar lowering capacity in diabetics which is from 5 to 40 times greater than tolbutamide; the action of chlorpropamide is stated to be from 2 to 10 times greater than that of tolbutamide.

General Considerations Relative to Sulfonylurea Therapy

The major problems in the treatment of diabetes are (1) control of the unstable diabetic, (2) management during acute stresses, and (3) prevention of diabetic "complications." Since the sulfonylureas are not very efficacious in the first two situations and we do not yet know much about their effectiveness in the last it appears that they leave much to be desired. On the other hand, experiences suggest that they have several advantages. Although they have been useful chiefly in patients over

40 years of age with stable and mild diabetes, this group contains hundreds of thousands in the United States alone. Moreover, a significant number in this group is handicapped in the insulin regimen by such things as visual or psychic disturbances. Because of the frequent existence of vascular complications in this group, hypoglycemic reactions should be avoided and this is much easier with the sulfonylureas than with insulin. However, more precautions along this line must be taken with chlorpropamide than with tolbutamide. As far as can be judged by the blood and urine sugar levels, patients previously treated with insulin and subsequently selected for sulfonylurea therapy have been as well controlled with the latter as with insulin. With larger doses of insulin there would have been less hyperglycemia but more hypoglycemia, a complication that must not be regarded lightly (Chap. 48). Most of the patients who have been inadequately treated with diet alone respond satisfactorily to sulfonylureas. No doubt better enforcement of dietary regimens would net better results, but when extensive efforts in this direction have failed it seems preferable to use additional measures, particularly when sulfonylurea therapy is simple and the side effects so few. However, this type of therapy can be associated with great difficulty if patients are not properly selected. A patient with unstable diabetes may upon substitution of sulfonylurea for insulin develop severe diabetic ketoacidosis and death within 1 or 2 days. Most patients who readily develop ketoacidosis are not good candidates for sulfonylurea treatment, but in a few instances the diabetes may be made less labile by using sulfonylurea in conjunction with insulin at a reduced dosage level. Most diabetics who are subjected to major surgery and those who have pneumonia or other severe stresses should be treated with insulin rather than sulfonylureas, because the needs for insulin under these circumstances are in excess of the quantity of endogenous insulin that can be made available. Many patients who are allergic to insulin should be desensitized and treated with it even though sulfonylureas may ordinarily control the hyperglycemia. Such patients might otherwise suddenly be subjected to severe stress where oral therapy would be inadequate, and too little time would be available to desensitize the patient to insulin. Depancreatized patients should be treated with insulin since they respond inadequately, if at all, to sulfonylureas.

Most diabetics over 40 years of age with mild stable disease have such a good response to sulfonylureas that it hardly seems worthwhile in this group to conduct an acute screening test. The latter is simple and should, however, be performed in patients who might readily develop ketoacidosis. Before initiating a therapeutic trial, appropriate

evaluations should be made of the diabetic status as well as of other aspects of the patient's health. The appropriate diet should be continued or instituted (Chap. 33). Insulin therapy can be discontinued abruptly when the *daily requirements are less than about 30 units.* With larger requirements it is desirable to reduce gradually the insulin dosage while administering sulfonylurea. The importance of general attention to the patient's hygiene and health should be borne in mind by the patient and his physician. Moreover, the patient should be told about the possible good and bad effects of sulfonylurea therapy.

During the first week of sulfonylurea treatment the patient should test his urine* four times daily for sugar and ketones and should report several times to the physician—at least by telephone; hospitalization is not indicated in most cases. Follow-up by the physician should be according to the indications in the individual subject. In most instances it is clear within a week whether or not he will respond satisfactorily to this type of therapy; certainly within a month this can be established, along with the optimal dosage regimen. Patients not responding significantly within this time should be changed to insulin treatment.

As discussed previously, approximately 5 per cent of patients who *initially have responded satisfactorily to tolbutamide subsequently fail* to experience significant benefit, and are said to have developed "secondary failure." Since a large number of such patients respond readily to chlorpropamide or metahexamide it seems probable that the failure may have resulted from an increased rate of metabolism of the drug in the body rather than a refractoriness of the β-cells to stimulation. Actually, some of the patients with secondary failure to tolbutamide not only have been found to respond to other sulfonylureas, but also have responded a few weeks later to tolbutamide. Indeed, rather than "exhausting" the β-cells, it is possible that the sulfonylureas may permit them to work more efficiently. Loubatieres and others claim that sulfonylureas actually have an "antidiabetic" effect. Animals partially depancreatized and treated with this therapy experience a lower incidence of diabetes. Some patients treated with sulfonylurea for several months require several weeks after cessation of treatment for reappearance of frank diabetes. Whereas in certain individuals the severity of diabetes becomes worse after a course of sulfonylurea therapy, careful study suggests that the treatment did not cause this. There are as many instances where there is amelioration of the diabetes, and in the majority there is no definite change. With poor control the diabetes may become

* Metahexamide and chlorpropamide, unlike some sulfonylureas, do not give a positive urine test for albumin.

worse, just as with inadequate insulin therapy; patients should not be permitted to continue under poor control. In conclusion, long-term sulfonylurea therapy has not been shown to damage the pancreas or other tissue, except in a few instances of hypersensitivity reactions, most of which have been transient. Whereas chlorpropamide and metahexamide exert a stronger and more prolonged blood sugar lowering effect than tolbutamide, the incidence of jaundice is sufficient to preclude use of metahexamide and to indicate chlorpropamide in diabetics with primary or secondary failure to tolbutamide and in other special instances.

THIADIAZOLES

The isopropylthiadiazole derivative of sulfanilamide was the first sulfonamide demonstrated to have marked hypoglycemic action. Its actions are apparently similar to those of the sulfonylureas. Many other thiadiazoles have been found to exert similar action. Altering the length of the alkyl chain of sulfamidothiadiazoles changes the hypoglycemic potency. The butyl radical is the most potent, while the amyl and propyl are less active. Substitution of a methyl radical for the amino in the 2-position does not modify the action appreciably but methoxy substitution greatly increases it; ethoxy substitution reduces it. 2-Methoxybenzenesulfonylamino-5-t-butylthiadiazole and 2-methoxybenzenesulfonylamino-5-isobutylthiadiazole (Fig. 35-1) have been found in preliminary studies in animals to exert comparable hypoglycemic action to tolbutamide. In rabbits, hypoglycemia following oral administration appeared in the second hour and was maximal at the fifth.

Clinical studies with methoxybenzenesulfonylaminoisobutylthiadiazole have shown it to be much less satisfactory than the sulfonylureas.

BIGUANIDES

Ungar discovered that phenethylbiguanide (PEBG, phenethylformamidinyliminourea, DBI) caused marked hypoglycemia in animals, and Pomeranze showed that it was effective in the management of some patients with diabetes. Decades earlier it had been demonstrated that guanidine produced hypoglycemia and that decamethylene diguanidine (synthalin A) exerted a marked hypoglycemic action. Indeed, synthalin was used in the treatment of many diabetics but later discontinued because of toxicity. The decamethylene radicals contribute greatly to the toxicity of this diguanidine. As seen in Figure 35-8, there is a difference in the structure of a diguanidine and a biguanide. Although this difference is small, it accounts for a significant difference in toxicity.

Paludrine, a biguanide used extensively in the treatment of malaria, has proved to have a relatively very low level of toxicity.

PEBG (Fig. 35-8) has been investigated in regard to its actions upon carbohydrate metabolism more than any of the other biguanides. It, along with amyl and isoamyl derivatives, is the main one that has been tested in diabetic patients. PEBG is a white crystalline powder that is soluble in water. It is rapidly absorbed whether administered orally or subcutaneously. If injected rapidly intravenously, it may cause

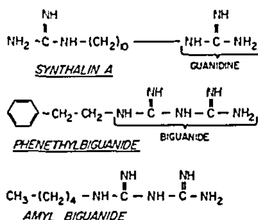


FIG. 35-8. Relation of structures of guanidine, decamethylene diguanidine (synthalin A), and biguanides. There are similarities but marked differences in hypoglycemic potency and in toxicity. (By permission, after ODELL, W. D., TANNER, D. C., STEINER, D F., and WILLIAMS, R. H *A.M A Arch. Int. Med. 102:520, 1958.*)

With inhibition of the oxidative enzymes a state of tissue anoxia is produced and this leads to hypoglycemia through two mechanisms (Fig. 35-9): (a) increase in glucose uptake by peripheral tissues (Pasteur effect), and (b) decrease in hepatic glucogenesis. The inhibition of respiration causes an increase in anaerobic glycolysis, with an increase in lactic acid production and a decrease in muscle glycogen. Anoxia inhibits gluconeogenesis and leads to a decrease in liver glycogen and a decrease in hepatic glucogenesis.

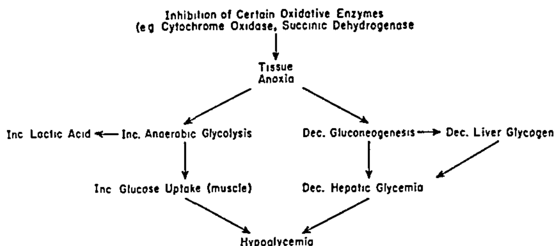


FIG. 35-9. As discussed in the text, there is evidence suggesting that PEBG inhibits oxidative phosphorylation; this could lead to anoxia. Conversely, anoxia can inhibit oxidative phosphorylation There is insufficient information for concluding which is primary. However, in either instance the changes shown in the above figure could be expected and have been demonstrated to occur under certain experimental conditions. (Modified after WILLIAMS, R. H., STEINER, D. F., ODELL, W. D., TANNER, D. C., and HENLEY, E. D.: *Diabetes Mellitus*, Third Congress of the International Diabetes Federation, Dusseldorf, July, 1958. Stuttgart, George Thieme Verlag, 1959.)

Not only pyruvate and lactate accumulate in the blood and disappear at a subnormal rate, but also citrate and other intermediates of the Krebs cycle. There is a decreased rate of lipogenesis and a decrease in the oxidation by adipose tissue of glucose, acetate, and succinate. Anaerobiosis and many compounds that inhibit oxidative phosphorylation increase glucose uptake and inhibit gluconeogenesis. However, insulin can stimulate the same amount of uptake under aerobic as under anaerobic conditions, moreover, insulin stimulates rather than inhibits oxidative phosphorylation.

PEBG is *like* insulin in the following regards:

1. Promotes hypoglycemia.
2. Increases glucose uptake by tissues.
3. Increases blood pyruvate and lactate after glucose administration.
4. Decreases gluconeogenesis.

PEBG is *unlike* insulin in the following ways:

1. Produces anoxia.
2. Does not increase glucose uptake when anoxia has been produced via other means.
3. Inhibits oxidative phosphorylation.
4. Inhibits cytochrome oxidase and succinic dehydrogenase.
5. Causes accumulation of products of Krebs cycle.
6. Causes decrease in rate of disappearance of pyruvate, lactate, and citrate.
7. Inhibits glycogenesis and decreases glycogen stores.
8. Decreases the oxidation of glucose, acetate, and succinate.
9. Increases blood phosphate.

Therefore, there are many ways in which PEBG does not act like insulin. Its presumable inhibition of oxidative phosphorylation, the decrease in high energy phosphate and the decrease in activity of the Krebs cycle could account for some of the late untoward effects that have been observed in some patients. Insulin has been found to reverse these untoward clinical effects rapidly and it can be expected to reverse many of the biochemical changes.

Many of the observations made with animals via in vivo or in vitro studies have not been found in man. The differences may be related less to species variation than to the use of relatively much smaller quantities of PEBG and to less detailed studies. Many of these same observations have been made with synthalin and other guanidines.

The biguanides cause hypoglycemia in the absence of the liver and/or the pancreas, but the effect is much greater in the presence of these organs. Creutzfeldt has found that PEBG promotes less degranulation and vacuolization of the α-cells than does synthalin. PEBG does not alter significantly the morphology of the β-cells and apparently does not influence directly either glucagon or insulin secretion

In studying approximately 100 diabetics treated with biguanides (phenethyl, amyl, and isoamyl derivatives), my colleagues and I have found that approximately half experienced a fall in fasting blood sugar by 30 per cent or more, with at least a 50 per cent decrease in glycosuria, or a decrease in insulin requirements by 30 per cent or more.

Some patients were better controlled with PEBG than with insulin or tolbutamide. There was no correlation of the response to therapy with age or duration of diabetes, but there was an inverse relationship to the previous dosage of insulin. Treatment was somewhat hindered by a high incidence of side effects of two types: (a) early, occurring usually within the first few days and consisting of anorexia, nausea, and vomiting, or (b) late, and consisting of lassitude, weakness, and slight weight loss, noted after one or more months of therapy. The early side effects were observed by us in approximately half of the patients and the late side effects in about one third of those treated for longer than one to two months. All side effects disappeared within 1 to 3 days after cessation of biguanide therapy. No permanent ill effects have been observed by us or reported by others. Krall observed side effects in one third of 121 patients. He found in 86 per cent of his patients a significant blood sugar lowering effect. "Good" or "fair" control was observed in 66 of 88 patients.

Of particular interest has been the results of biguanide therapy in juvenile diabetics. Since Krall has had the most extensive experience along this line, his studies are summarized. Seventy-two patients with growth-onset diabetes were treated with either PEBG or the amyl derivative. The results were as follows: 35 successful, 29 discontinued, 8 failures. In 89 per cent a blood sugar lowering effect was observed, but in 53 per cent there were either side effects or failure to lower the blood sugar. Thirty of these juveniles were treated for an average of 7 months: 16 with insulin in addition and 14 without insulin. The average daily dose of insulin used was 10 units, which constituted a 64 per cent reduction of the usual dosage. The average daily dose of biguanide was 152 mg. for those who also received insulin and 110 mg. for the others. The best results were obtained in those with diabetes of most recent onset. Gastrointestinal symptoms developed in half of the cases but were not always severe enough to cause discontinuation of therapy. There was no severe hypoglycemia and no renal, hepatic, hematologic, or other complications occurred. Linear growth and weight gain proceeded at normal rates. Recent diabetes with biguanide alone did better than expected in delaying natural intensification of diabetes and the long-term cases did better than expected with respect to insulin requirements. Krall concluded: an attempt at stabilization is justified in the usually labile, severe growth-onset diabetic. When side effects can be eliminated, biguanide appears to be a useful adjunct in the total therapy of juvenile diabetics.

Considering all the facts available, it may be stated that biguanides apparently are useful in the treatment of some diabetic patients. The

high incidence of gastrointestinal symptoms precludes extensive use. These probably are chiefly of central origin and dextroamphetamine has been said to reduce their frequency significantly. Biguanides have apparently promoted a smoother control of juvenile diabetes, reducing significantly the amount of hyperglycemia, hypoglycemia, and insulin dosage. In this regard they are superior to sulfonylureas. In some instances of maturity-onset diabetes they have been more effective in maintaining euglycemia than either insulin or sulfonylureas. However, the sulfonylureas are favored in the majority of stable diabetics because of the much lower incidence of side effects and because of the more normal physiologic changes induced. PEBG and the amyl derivative seem to have a comparable capacity to reduce hyperglycemia and the incidence of side effects is similar. The isoamyl derivative is less effective than either of the other two in controlling the diabetes and its use is not recommended. In most instances it is preferable not to use more than 200 mg. daily of PEBG or of the amyl derivative. In some cases, however, much larger doses are given over long intervals without any side effects. Patients who develop the side effects usually do so within the first few days. Late side effects rapidly disappear upon substituting insulin therapy. No significant side effects other than the ones mentioned have been observed, all have reversed within a few days. The smaller the dosage the less frequent the side effects, but the severer the diabetes the larger the dosage required for adequate control. PEBG used simultaneously with one of the sulfonylureas is sometimes more efficacious than either drug alone. Since they act in different ways the effects tend to be additive.

OTHER COMPOUNDS

The hypoglycemic action of a large number of other compounds has been studied. Many of these have been reviewed by Lewis, Mirsky, Goldner, Williams, and others. Benefit in the treatment of diabetes has been claimed for a large variety of compounds, including extracts of molds, yeast, and bacteria, and of the roots, stems, leaves and berries of certain plants. Investigations have included acridines, penicillin, thiouracil, amellin, galigen, myrtillin, lupanine, pancreatic enzymes, tolazoline (Priscoline), tripelennamine (Pyribenzamine), benzyldimethylphenylethylenediamine (Antergan), antazoline (Antistine), salicylates, estrogens, peptides, amino acids, auxins, corchorus olitorius, aspergillin, nicotinic acid, mesoxalic acid, 2-amino-methylenecyclopropanepropionic acid (Hypoglycin A) and many others. Because of lack of potency, or frequency or severity of side effects, none of these offers promise in the

treatment of diabetes. In searching for compounds that may be useful in treating this disease it must be borne in mind that knowledge of their effect on blood and urine sugar concentrations is desired, but additional information is needed. The major objective is to correct *all* the abnormalities in carbohydrate, fat, and protein metabolism. It is possible to lower the blood sugar yet not increase the utilization of any food; this is not of significant benefit.

SUMMARY

Treatment of diabetes with oral therapy constitutes a highly desirable goal. Thus far two sulfonylureas have offered the greatest promise: tolbutamide and chlorpropamide. They apparently decrease hyperglycemia chiefly by stimulating an increased supply of insulin, promoting increased glucose utilization. They also seem to decrease hepatic glucogenesis by increasing the insulin supply and by acting directly upon the liver. The last effect is relatively weak, but appears to be intensified via permissive insulin action. The sulfonylureas are most effective in subjects who most approach normalcy in the assayable insulin of the pancreas and plasma. These tend to be elderly individuals with stable diabetes of recent onset. Depancreatized subjects, those with unstable diabetes (juvenile-onset), those of long duration, those requiring large insulin doses, and those readily prone to develop ketoacidosis, respond poorly or not at all. Certain selected groups can be anticipated to have as high as a 90 per cent incidence of favorable responses whereas others will have less than 10 per cent. There is no evidence that prolonged sulfonylurea therapy leads to exhaustion or a refractoriness of the β-cells; indeed, the cells may actually be led to function more efficiently. Side effects have been rare and in most instances mild. Chlorpropamide has yielded a higher incidence of favorable responses than tolbutamide, probably because of a longer half-life in the body. Since, however, chlorpropamide occasionally produces jaundice tolbutamide is recommended more often. Chlorpropamide is used in diabetics with primary or secondary failure to tolbutamide, as well as in other select cases. Metahexamide is no longer recommended because of the higher incidence of jaundice associated with it.

Phenethylbiguanide and amylbiguanide have approximately equal merit, each is superior to the isoamyl derivative. These compounds apparently act by inhibiting certain oxidative enzymes of the Krebs cycle, particularly succinic dehydrogenase and cytochrome oxidase. This leads to (a) an increase in anaerobic glycolysis with an increase in glucose uptake, and (b) a decrease in gluconeogenesis, decreased liver glycogen,

and decreased hepatic glucogenesis. Thus there is a decrease in the amount of glucose contributed to the blood and an increased rate of transfer of glucose from blood to tissue. The biguanides lower the blood sugar in all types of diabetics, but the greatest effect is manifested in the mild, stable type. These drugs have led to a smoother control of juvenile diabetes, either when given alone or in conjunction with a reduced dosage of insulin. In some patients biguanides have been administered concomitantly with one of the sulfonylureas, causing better control than either drug alone. The great frequency of early side effects (anorexia, nausea, vomiting) has significantly curtailed the use of this type of therapy. Moreover, some of the patients have developed disconcerting late effects (malaise, weakness, weight loss). All side effects have completely disappeared within a few days, usually with discontinuation of the drug.

A vast number of potentially effective antidiabetic compounds have been studied in the past and the number available for investigation is increasing at a rapid rate. These must be evaluated objectively, observing the effects not only on the levels of blood and urine sugar but upon many phases of the metabolism of carbohydrates, fats, and proteins, and also upon the general health of the patients over many years.

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Chapter 36

MANAGEMENT OF DIABETES DURING STRESS AND SURGERY

Peter H. Forsham

The management of diabetes is concerned with the maintenance of as constant an internal environment as possible at all times. This is achieved by balancing the intake of food against requirements for its utilization, with or without the aid of appropriate hypoglycemic agents. Any stressful event will interfere with this balance.

STRESS

During a period of stress, carbohydrate tolerance may be sharply decreased by an increased secretory activity of the sympathetic system, notably of epinephrine, which increases the rate of glycogenolysis and hence the discharge of glucose from the liver. A concurrent rise in glucocorticoid secretion by the adrenal cortices will lead to an increased rate of gluconeogenesis from protein and fat and in conjunction with diabetogenic activity of the pituitary the peripheral anti-insulin effect will be increased. As a rough but useful approximation, it may be stated that the administration of 5 mg. of hydrocortisone to a juvenile diabetic, practically devoid of insulin, will increase insulin requirements

by 10 units. Inasmuch as up to 250 mg. of hydrocortisone per day may be secreted by the adrenals under extreme stress, a maximum rise in insulin requirement of 500 units would be the theoretical ceiling. In certain forms of chronic stress, notably those involving extreme cold, increased secretion of thyroid hormone will cause a constant shift of carbohydrate from the liver to the periphery, secondarily elevating blood sugar levels.

It is apparent that all these factors will, to a varying extent, lead to a deterioration of diabetic control. A reactive hypoglycemia might develop following the period of acute stress in patients with prematurity onset of diabetes receiving insulin or with the adult type of diabetes with varying reserves of insulin that can be secreted under certain circumstances.

Emotional Stress

In the management of prematurity onset diabetes with diet and insulin, emotional stresses usually upset the regulation. It is imperative, however, not to increase the dosage of insulin, if at all possible, except where a dangerous state of ketoacidosis develops. For if one allows the patient to increase the insulin dosage during periods of psychological upheaval, then periods of hypoglycemia usually follow and these call forth insulin antagonists to an increased extent. This in turn sets up a vicious circle whereby more insulin is usually taken and this in turn produces another set of hypoglycemic episodes with further rise of the insulin antagonists. In this manner patients may be given 5 to 10 times the dose of insulin that would actually control their blood sugar levels under ordinary circumstances. Even at the risk of having a few days of excessive hyperglycemia, one should not increase the insulin at all or only to a very slight extent, and always be ready to reduce it again to previously satisfactory levels as the emotional problems decrease in intensity. In the patient with maturity onset of diabetes the impact of emotional stresses on the disease is not as violent as in the juvenile diabetic, but even here hypoglycemic agents, whatever they might be, should not be increased unless absolutely essential. The liberal use of tranquilizers is most helpful in minimizing wide surveys in blood sugar levels.

Physical Stress

This will lead to hyperglycemia only when it affects the organism during a period of relative physical rest. If much exercise is associated with the physical stress, the dangers of hypoglycemia far outweigh those of hyperglycemia. The lighthouse keeper who failed to reach his light-

house in a row boat on a stormy morning is a case in point. He took the same amount of insulin as when settled for an 8-hour day in his lookout tower and consequently fell victim to a hypoglycemic reaction. Patients who anticipate periods of heavy exercise or moderate stress with exercise must be told to reduce their insulin intake by one third to one half or more, and if this is not foreseen, to eat more during and after the exercise. The same principles should be followed with sulfonylurea therapy. It is of importance to recognize that whether a patient is receiving long-acting insulin or a long-acting sulfonylurea, such as chlorpropamide, the danger of insidious hypoglycemia during stress is even greater than with the more rapidly acting crystalline insulin preparations or tolbutamide.

INFECTION

The diabetic is more subject to infection generally than other patients. Since any infection represents a more or less severe stress, hyperglycemia will be produced through the mechanisms previously discussed. In patients on insulin therapy who invariably show some degree of binding of injected insulin to serum proteins, this phenomenon may be increased through a nonspecific anamnestic immune response that would decrease the effect of the insulin. Thus adequate diabetic regulation during infections demands an early increase in the insulin dosage. Cases on oral hypoglycemic therapy should be given supplementary insulin during the active phase of the infection. The appearance of ketonuria makes this particularly necessary. The diabetic appears to have a less efficient defensive response to infectious agents than a normal subject and thus the early and often prolonged use of antibiotics, preferably those of the bacteriocidal rather than the bacteriostatic variety, are called for.

SURGERY

With major surgical procedures there is a specific type of stress to the diabetic that requires special management dependent on whether or not there has to be much curtailment of food intake. If not, as with minor surgical procedures under local anesthesia, one hardly needs to alter the everyday management. With major surgery and general anesthesia in a patient treated with insulin or oral hypoglycemic agents, surgery is to be delayed except in an emergency, so as to obtain control of all aspects of the diabetic state, including glycosuria, hyperglycemia, dehydration, ketoacidosis, and depleted glycogen and vitamin stores. This may be achieved best by giving a diet containing at least 250 gm. or carbohydrate for at least two or three days preceding surgery.

The *anesthesia* selected should be one that minimizes anoxia and acidosis and thus least disturbs carbohydrate metabolism. In this respect local anesthesia is preferred, but spinal anesthesia is satisfactory; cyclopropane, nitrous oxide, and ethylene are relatively safe. Intravenous Pentothal Sodium should be used cautiously and only for short periods, while ether is undesirable and chloroform and Avertin are definitely contraindicated.

Preparation for and *treatment* during and after surgery should aim at allowing the diabetic patient to utilize enough carbohydrate to prevent ketoacidosis, which requires from 800 to 1,200 calories derived from carbohydrate per day. *Patients treated with insulin* should be given one half the usual dose of long-acting insulin preparation on the day before surgery so as to prevent hypoglycemia after a prolonged preoperative fast. If the patient receives crystalline insulin, any dose within 12 hours before surgery should be halved. For patients receiving *oral antidiabetic treatment*, the evening dosage should be omitted and the patient should be given insulin therapy until he is able to take food by mouth.

On the morning of surgery one half the usual dose of insulin is administered subcutaneously prior to starting an infusion of 1,000 ml. of 10 per cent dextrose in water at a slow rate (20 to 30 drops per minute). From 2,000 to 3,000 ml. are given on the day of surgery and during subsequent days if there is inadequate oral food intake.

It is desirable to maintain the blood sugar within reasonable limits and to be satisfied with a mean urine sugar reduction of 1 to 2+. One should not aim at any stricter control, since the danger from hypoglycemia far outweighs that of a slight hyperglycemia. If the patient is able to urinate spontaneously, one should determine urine sugars every 8 hours and administer crystalline insulin in minimum doses of 20 units for 4+ reduction, 15 units for 3+ reduction, and 10 units for 2+ reduction. If urine cannot be obtained spontaneously one should not catheterize the diabetic patient because of the great danger of starting intractable urinary infections. Instead, a blood sugar determination at 8 A.M. and 5 P.M. may be used to gauge the insulin requirements. As a rule of thumb, one administers a minimum of 10 units of crystalline insulin every 8 hours for every 50 mg elevation of blood sugar above 150 mg. per 100 milliliters. Since a considerable amount of glucose may be lost in the urine when intravenous infusions are administered too rapidly, this method of control may be preferable to that based on fractional urine sugar determinations. The addition of sodium and potassium chloride or sodium lactate as well as potassium chloride will be governed by the presence or absence of ketoacidosis and prevailing electrolyte losses.

During the *postoperative period* the oral intake of food should be commenced early with orange juice and clear soups. At that time either long-acting insulin preparations or oral antidiabetic agents should be resumed in lower than the usual doses and should then be rapidly increased if necessary up to the preoperative level. Since many diabetics have an increased capillary fragility and a reduced facility for wound healing, supplementation with ascorbic acid (300 to 500 mg. per day), pyridoxin (50 to 100 mg. per day) and the administration of anabolic hormones should be seriously considered. A preoperative check on the prothrombin time affords an important safeguard.

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Chapter 37

DIABETIC ACIDOSIS

William H. Daughaday

Diabetic coma remains one of the most dramatic metabolic disturbances encountered in clinical medicine. The far-reaching implications of this condition have been a continuing stimulus for research in carbohydrate, fat, protein, and body fluid, and electrolyte metabolism. The results of these investigations have led to major improvements in treatment with the result that death from diabetic acidosis in well-conducted clinics has become rare.

PATHOLOGIC PHYSIOLOGY

Nearly all the clinical features of diabetic ketoacidosis can be attributed to two interrelated disturbances. The first of these is the accumulation of glucose in the blood and the second is the overproduction of β-oxybutyric acids.

Hyperglycemia

The fundamental biochemical lesions in carbohydrate metabolism of diabetes have been discussed in Chapter 9. In general terms, glucose

utilization becomes insufficient in the absence of insulin because of the decreased penetration of glucose through the cellular and intracellular barriers that are interposed between the glucose of the extracellular fluids and the active site on the enzyme glucokinase. Despite the great amount of work stimulated by Cori's suggestion that the glucokinase activity in diabetes is inhibited, there is little definitive information on this possibility.

Impaired peripheral utilization of glucose is only one factor leading to hyperglycemia. There is now abundant evidence that hepatic glucose overproduction makes a major contribution to hyperglycemia. Direct evidence of hepatic glucose overproduction in diabetic acidosis has been obtained by measuring splanchnic glucose production by hepatic vein catheterization (5). One explanation for glucose overproduction by the liver is provided by important observations in experimental diabetic rats by Ashmore, Hastings, and Nesbett (2). The level of glucose-6-phosphatase was found to be greatly elevated in the diabetic state. This enzyme, which hydrolyzes glucose-6-phosphate to free glucose, is crucially concerned with the release of glucose by the liver.

Hyperglycemia, as generated by decreased glucose utilization and increased hepatic gluconeogenesis, leads to some of the damaging effects of uncontrolled diabetes. The predominantly extracellular location of glucose means that the osmolarity of the extracellular fluids rises with hyperglycemia. Because of the osmotic gradient, water leaves the intracellular compartment (*intracellular dehydration*) to enter the extracellular compartment.

A tremendous increase in the glucose entering the renal glomerular filtrate is a second important result of hyperglycemia. Normally only about 100 to 200 mg. of glucose per minute are filtered but in diabetic acidosis this may rise to values five to ten times that amount. The ability of the proximal convoluted portion of all the nephrons to remove glucose is limited to about 300 to 350 mg. per minute. The remaining glucose that passes down the tubule impairs seriously the reabsorption of water and electrolytes from the tubular urine. A careful study of glucose diuresis has been made by Brodsky, Rapoport, and West. It was found that urine flow was related to the total osmotic load. Another characteristic of an osmotic diuresis deserves emphasis. Water conservation is achieved in simple hydropenia at a maximal urine concentration of 1,200 to 1,400 milliosmols per liter. With the increased urine flow of an osmotic diuresis the ability of distal tubule and collecting ducts to reabsorb water is overtaxed and the maximal urinary concentration falls to about half of that achieved in the simple hydropenic subject. The large volume of urine that escapes reabsorption in the proxi-

mal tubule exceeds the *capacity of the concentrating mechanisms* of the distal tubule.

The degree of hyperglycemia may be an indication of abnormal renal function. In nonacidotic diabetic patients who are well hydrated, it is unusual to find blood sugar values over 500 mg. per cent because intense glycosuria inhibits further glucose accumulation. Higher blood glucose concentrations are common in severe diabetic acidosis, partly because the excretion of glucose has fallen, the result of a fall in the glomerular filtration rate.

Unlike the diuresis that occurs in diabetes insipidus, the consequences of the osmotic diuresis of diabetes mellitus are not limited solely to water loss. Even in the absence of acidosis, increased amounts of sodium, potassium, and other electrolytes are excreted in the urine. Glucose diuresis in one of the diabetic patients studied by Brodsky, *et al.*, led to a sodium loss in the urine of 1.5 mEq. per minute. This loss, if sustained, would lead to the depletion of virtually the entire extracellular fluid within 24 hours. The important role of glycosuria in leading to electrolyte deficits in the absence of ketosis is apparent in one case of uncontrolled diabetes studied by Atchley and co-workers (3) over a longer period of time following the withholding of insulin. Large quantities of sodium, potassium, chloride, and phosphate were lost in the urine as a consequence of severe glycosuria although ketosis never developed.

β-Oxybutyric Acid Overproduction and the Problem of Metabolic Acidosis

The accelerated breakdown of fatty acids in uncontrolled diabetes, discussed in Chapters 10 and 13, leads to the outpouring of large amounts of acetoacetic acid and β-hydroxybutyric acid by the liver. Acetone is formed to a limited extent by the spontaneous decarboxylation of acetoacetic acid. The total quantities of these acids which are produced may be very large. Although more than 40 gm. of ketone bodies may appear in the urine daily, hepatic production may far exceed urinary excretion. The urinary excretion of ketoacids in diabetic cats studied by Stadie, Zapp, and Lukens was found to be only about one seventh of the ketoacid production as estimated by several indirect methods. The utilization of acetoacetic and β-hydroxybutyric acids by the tissues accounts for the difference between production and urinary excretion. Although it is commonly held that the diabetic animal can utilize ketone bodies normally, recent experiments with diaphragms and skeletal tissues from diabetic rats have established a moderate impairment of oxidation of these acids (4).

The metabolic consequences of the accumulation of β-oxybutyric acids* can be attributed to the separate effects of the hydrogen ion and the organic anion. Attention generally has been focused on the aceto-acetate or β-hydroxybutyrate anions but conceptually it is preferable to consider the metabolic acidosis as due to hydrogen ion excess. Emphasis on the primacy of hydrogen ion in understanding the pathologic physiology of acidosis is advocated by Christensen.

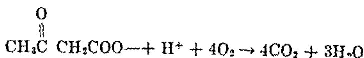
The fate of the hydrogen ions that enter the body fluids in diabetic acidosis must be clearly understood. In the extracellular fluids, hydrogen ion combines with bicarbonate ion in the following familiar sequence of reactions:



$$H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2\uparrow + H_2O$$

The effect of the addition of hydrogen ion to this system is to drive the reaction to the right, lowering serum $HCO_3^=$ and increasing the partial pressure of carbon dioxide, but with only a slight rise in H^+ ion concentration. These chemical alterations in the blood stimulate the chemosensitive cells of respiratory center to increase respiratory effort, manifested clinically by the very deep and rapid Kussmaul respirations of diabetic acidosis. As shown in Figure 37-1, there is an important relationship between the arterial pH and the ventilatory volume. It is noteworthy that Kussmaul respirations are not generally observed at pH values above 7.2. Respiratory effort may decrease secondarily in very ill patients because of muscular fatigue or respiratory center narcosis. When this occurs there is a progressive rise in hydrogen ion concentration of grave prognostic significance.

The buffering effect of the bicarbonate system and the related respiratory lowering of the partial pressure of carbon dioxide minimize the changes in the extracellular fluid hydrogen ion concentration but do not rid the body of excess hydrogen ion. To reconstitute the normal electrolyte pattern of serum the reactions must be reversed and hydrogen ion will reappear. For this reason the ultimate correction of the hydrogen ion excess depends upon either (1) the oxidation of the organic oxybutyric acids,



$$CH_3\overset{\overset{O}{\|}}{C}\ CH_2COO^- + H^+ + 4O_2 \rightarrow 4CO_2 + 3H_2O$$

or (2) the renal excretion of the hydrogen ion.

* β-Oxybutyric acid is used as a generic term to include acetoacetic acid and β-hydroxybutyric acids.

The renal excretion of hydrogen ion is accomplished in two ways: (1) the preferential exchange of H^+ for sodium by the renal tubule, (2) the excretion of ammonia by the renal tubule that reacts with hydrogen ion to form the ammonium ion. The kidney, *therefore, is a*

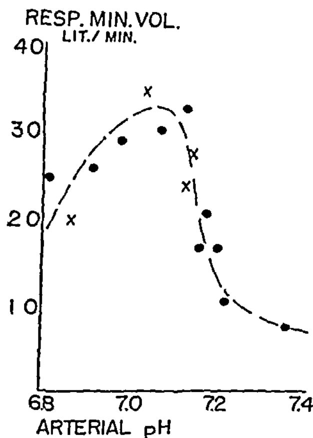


FIG. 37-1. The pulmonary ventilation in diabetic acidosis has been plotted as a function of the arterial pH. The circles are observations on patients who ultimately recovered; the crosses on those who succumbed (*From* Kety, S. S., Polis, B. C., Nadler, C. S., and Schmidt, C. F.: *J. Clint. Invest.* 27:500, 1948)

key organ in protecting the body against the harmful effects of a rising hydrogen ion concentration. The hydrogen ion balance in diabetic acidosis is the difference between hydrogen ion production, and hydrogen ion utilization by oxidation and excretion. As the utilization of the β-oxybutyric acids may be limited to the equivalent of 1500 calories

per day the balance of the hydrogen ion load must be excreted if the patient is to remain in any type of relatively satisfactory equilibrium.

Any impairment in the ability of the kidney to excrete hydrogen ion might be expected to accelerate the development of diabetic acidosis. For this reason it is surprising that organic renal disease is not a more common predisposing factor in the production of diabetic acidosis. This may be attributable to the amelioration of the diabetic state that usually occurs in diabetic nephropathy.

Functional renal impairment, however, is an important feature of nearly all cases of severe diabetic acidosis. Dehydration leads to decreased glomerular filtration and renal plasma flow. Ammonia production is limited by the decreased rate at which ammonia precursors (primarily glutamine and amino acids) reach the renal tubule cell for deamination. Clarke and co-workers have pointed out that the urinary pH is usually higher in diabetic acidosis than occurs in simple ammonium chloride acidosis of comparable severity (Table 37-1). This

TABLE 37-1. IMPAIRMENT IN HYDROGEN ION EXCRETION* IN DIABETIC ACIDOSIS

	Predicted†	*Observed*‡
Urine pH	4.7	5.4
H^+ excreted as titratable acidity	305	151
With phosphates	(71)	(68)
With ketoacids	(150)	(48)
With other organic acids	(84)	(35)
H^+ excreted as NH_4^+	>250	227
Total H^+ excreted	>555	378

* H^+ excretion in mEq /day.
† Hydrogen ion excretion which would be predicted if pH of urine had been lowered to pH 4 7.
‡ Data of Atchley, *et al* (3), recalculated by Clarke, *et al.* (12).

failure in hydrogen ion secretion was attributed by these authors to a depletion of renal cellular potassium. A vicious circle rapidly develops in which acidosis promotes sodium and potassium depletion and this in turn further impairs hydrogen ion excretion.

Hydrogen ion excess contributes in an important way to the clinical picture of diabetic acidosis in provoking hyperventilation and the loss of electrolytes in the urine. Some of the losses of intracellular electrolytes (K, Mg, and P) can be directly attributed to acidosis. The cell in an environment of increased hydrogen ion concentration maintains

intracellular-extracellular gradients with difficulty owing to faulty operation of the ion transfer mechanisms. Moreover, a lowering of intracellular pH releases bound potassium and magnesium and promotes the hydrolysis of certain organic phosphates. In addition, acidosis promotes the mobilization of bone minerals. It has been shown that bone sodium contributes to a significant degree in decreasing the depletion of extracellular fluid cations in experimental diabetic acidosis.

The abnormalities in renal function in diabetic acidosis help explain some of the clinical peculiarities observed in this condition. Many patients give evidence of having gone for days or weeks with a compensated ketosis without the development of frank acidosis. This compensation, however, is easily upset if nausea and vomiting supervene with failure of fluid intake. Dehydration leads to a falling rate of glomerular filtration and H^+ ion excretion. Thereafter the spiral into severe diabetic acidosis may occur with dramatic suddenness.

Water and Electrolyte Balances

The body fluid deficits incurred in diabetic acidosis are the sequelae of the osmotic diuresis and of the limitations of hydrogen ion utilization and excretion. Metabolic balance studies have yielded important information concerning the magnitudes of the losses of water and the various electrolytes from the body. The entire sequence of changes occurring in the development of ketoacidosis has been allowed to take place in a limited number of diabetic subjects while under complete metabolic balance measurements (3, 10). The meticulous observations of Atchley, Loeb, Richards, Benedict, and Driscoll of one such patient deserve particular mention. After the appearance of serious symptoms of diabetic acidosis, treatment was started and the retention of water and electrolytes during recovery was measured. Many diabetic patients in acidosis have been observed only in the recovery phase of their illness (13, 26, 30). These latter studies are open to the criticism that the retention of salts and water administered during treatment may not provide an accurate indication of the original losses because they may be influenced by the type of treatment employed. The various estimates of water and electrolyte losses are compared in Table 37-2.

These data indicate that, whereas the loss of total body water is in the neighborhood of 10 per cent of the body weight, only about half of this deficit arises from the extracellular fluid as judged by the negative balances for sodium and chloride. Intracellular electrolytes are lost in about the same amounts as the extracellular electrolytes. The fact that the potassium losses exceed that predictable by the negative nitrogen balance deserves special emphasis.

TABLE 37-2. COMPARISON OF ESTIMATED FLUID AND ELECTROLYTE REQUIREMENT IN DIABETIC ACIDOSIS*

Author	*Type of study*	*No. of patients*	*Na^+ (mEq./kg.)*	*Cl^- (mEq./kg.)*	*HCO_3^- (mEq./kg.)*	*K^+ (mEq./kg.)*	*Mg^{++} (mEq./kg.)*	*P (mM/kg.)*	*H_2O (L./kg.)*
Atchley, *et al* (3)	Loss during insulin withdrawal	2	5.9	2.5		4.9	. . .	. . .	0.089
Darrow (14)	Retention during acute therapy of diabetic acidosis and for 2 days after	1	13.3	9		6.1	. .	. . .	0.111
Butler, *et al.* (10)	Loss during insulin withdrawal	1	5.1	4.0	.	5.6	0.8	1.3	. . .
Butler†	Estimated loss 10 per cent dehydration	Estimated	5–12	4.0	. .	6.0	. .	. . .	0.06–0.1
Danowski, *et al* (13)	Retention during acute therapy of diabetic acidosis and up to 34 hours after acute therapy	8	10.4	9.5	. . .	6.0	. .	.	. . .
Nabarro, *et al.* (30)	Retention during therapy of diabetic acidosis and for 8 to 12 days after acute therapy on measured intake	7	7.2	5.1	. . .	5.0	0.56	0.5	0.087
Martin, *et al.* (26)	Retention during 12 hours of therapy of diabetic acidosis†	8	7.0	4.0	4.7	2.7‡	0.16‡	1.0‡	0.082

* Table from Martin, *et al* (26).
† Figures might well be higher as the authors divided retention by 70 kg. and this may not have represented average weight.
‡ This figure is too low for complete repair and represents amount for acute therapy only.

Lipid Changes Associated with Diabetic Acidosis

An elevation of all the major serum lipid components including the nonesterified fatty acids is present in diabetic acidosis. The increase in triglycerides is particularly striking. Frequently the serum has a lactescence due to large fatty particles of the size and density of chylomicrons. An occasional diabetic patient in acidosis will have lipemia of truly extraordinary proportions. Serum lipid levels between 10 and 20 per cent occur in these patients and even higher values have been reported.

The lipemia must be due to a mobilization of lipids from the fatty depots because hepatic lipogenesis is halted in uncontrolled diabetes. Certain pituitary proteins have marked fat mobilizing properties but the exact physiologic role of these hormones in diabetic acidosis is unknown.

Acidotic children exhibit hyperlipemia more commonly than adults but there is little correlation between the degree of acidosis and the extent of the lipemia. Some of the patients with extraordinary degrees of lipemia probably possess the hereditary trait of familial hyperlipemia in a homozygous or heterozygous state.

Lipemia may be recognized at the bedside by finding either lipemia retinalis or more rarely eruptive xanthomata. The presence of large quantities of fats in the serum leads to a significant reduction of serum water by simple displacement (1). An apparent decrease of the concentration of electrolytes and other solutes in whole serum may result. Considerably higher values would be obtained if the calculations were based on the concentration of solute in serum water.

Central Nervous System Function in Diabetic Acidosis

A depression of central nervous system function of the utmost clinical importance occurs in diabetic acidosis and may be associated with stupor and coma. It has been recognized in many clinical studies that prognosis is greatly influenced by the presence or absence of coma. The exact mechanism of the disturbance in cerebral function is disputed. The circulatory and metabolic changes in the brain have been measured by Kety, Polis, Nadler, and Schmidt. Mental state was correlated with the cerebral blood flow and oxygen consumption in diabetic patients using very ingenious indirect methods of measuring cerebral circulation. Cerebral oxygen utilization was clearly depressed (below 2.1 cc. per 100 grams of brain per [illegible] ute) in seven o[illegible] atients with diabetic coma. In comp[illegible] ious patients [illegible] tic acidosis utilized more than 2.4 [illegible] grams of br[illegible] inute (Fig.

37-2). The depression in cerebral oxygen metabolism was not attributable to a decrease in cerebral blood flow; actually cerebral circulation increased in diabetic acidosis.

Multiple biochemical abnormalities contribute to the depressed rate of oxygen utilization. There does not seem to be any decrease in

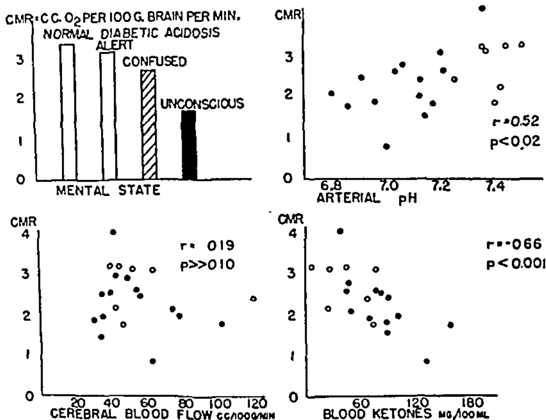


FIG. 37-2. Alterations in cerebral oxygen metabolism in diabetic acidosis. Cerebral oxygen consumption (CMR) has been correlated with mental state, cerebral blood flow, arterial pH, and arterial ketone concentration. Results obtained at hospital entry are indicated by closed circles; later observations are indicated by open circles. (*From* Kety, S. S., Polis, B. C., Nadler, C. S., and Schmidt, C. F.: *J. Clin. Invest.* 27:500, 1948.)

glucose utilization by brain in uncomplicated diabetes and insulin has not been shown to act upon neural tissues. A much greater acidosis can be induced experimentally in man without producing coma by administering large amounts of ammonium chloride. The possibility that the β-oxybutyric acids depress cerebral function directly has been subjected to much experimental study (17). Nearly all reports indicate that acetone and β-hydroxybutyric acid are not toxic in the concentrations found in the blood during diabetic acidosis. Serious consideration has

been given to the toxicity of acetoacetate. Intravenous infusions of acetoacetate have produced coma in experimental animals under conditions in which an equivalent amount of hydrochloric or β-hydroxybutyric acid would not produce coma. Kety and co-workers found that cerebral oxygen utilization was inversely related with the level of blood ketones. A less obvious correlation existed between arterial pH and the state of consciousness. The pathogenic role of acetoacetate in clinical diabetic coma cannot be completely accepted because acetoacetate produces coma experimentally only at blood levels that are higher than those observed in patients. The possibility that hitherto unrecognized products of metabolism are responsible for diabetic coma cannot be ignored. The synergism between acetoacetate levels, acidosis, and dehydration would appear to be the most likely explanation for the depression in cerebral oxidative metabolism.

Endocrine Function in Diabetic Acidosis

Adrenal cortical function in diabetic acidosis has been examined carefully by McArthur and her co-workers (27, 28). Six patients with severe diabetic acidosis were found to have increased amounts of adrenal metabolites in the urine. The omission of insulin in depancreatized dogs gave rise to increased adrenal activity. These observations of McArthur have been confirmed by Wallach, Englert, and Brown, who demonstrated that the plasma-free and conjugated 17-hydroxycorticosteroids were elevated in diabetic acidosis as well as the urinary corticosteroid metabolites. In severe diabetic acidosis, urinary 17-hydroxycorticosteroid excretion may be little elevated because of failing renal function. Adrenal hyperactivity does not appear to be an early result of insulin withdrawal but only occurs after significant ketosis has appeared. Nonetheless, adrenal hyperactivity may contribute to the physiological disturbances of acidosis by increasing insulin resistance, promoting protein breakdown, and stimulating the loss of potassium from cells in excess of nitrogen.

CLINICAL CHARACTERISTICS

Frequency

The incidence of diabetic ketoacidosis cannot be expressed in any meaningful way. The frequency of this condition among hospital admissions reflects the general level of medical care in the community and the spectrum of clinical interest of the attending staff. Where medical care is readily available and interest in diabetes is high, acidosis is rare. The occurrence of diabetic acidosis in a recognized case should

be looked upon as a serious failure in diabetic education or lapse in doctor-to-patient interrelationship.

Age and Sex

Diabetic patients who develop ketoacidosis are on the average younger than the general diabetic population. The mean age of four series, including 801 patients, collected by Joslin (22), was 29.3 years. This predominance of younger patients is attributable to the characteristics of juvenile onset diabetes as compared to adult onset diabetes. Diabetic coma in adult onset diabetes is unusual in the absence of an obvious precipitating cause such as infection or operation, but diabetes appearing in early life may progress rapidly to coma before the diagnosis is established. The need for exogenous insulin in most juvenile diabetics is absolute and acidosis develops after omission of insulin therapy.

A predominance of female patients greater than occurs in the diabetic population of comparable age exists in most surveys of diabetic acidosis. This observation can be attributed to a difference in ketogenesis in the sexes. The formation of ketone bodies during starvation is greater in women than in men. The tendency toward ketosis is particularly marked during pregnancy, where slight ketonuria may occur in well-regulated diabetics even during an overnight fast.

Precipitating Factors

Inadequate therapy will lead to acidosis in most young diabetics and in a minority of maturity onset diabetics. It has been suggested that irregularities of diet exceed any other cause of acidosis but, from the observations of Mirsky and co-workers it is doubtful that excessive dietary intake of carbohydrate will produce ketosis directly, although it will aggravate glycosuria with attendant fluid and electrolyte losses. Underutilization of glucose rather than overavailability of glucose is the critical factor leading to β-oxybutyric acid production. When carbohydrate utilization has fallen to a critical level, decreased food intake may accelerate ketosis.

Relative insulin insufficiency exists in all patients with diabetic acidosis, and the onset of this state is frequently the result of insulin omission or obvious errors in insulin administration. Careless patients may omit insulin entirely, or inadvertently switch from U-80 to U-40 insulin. A change in insulin requirement may not be recognized because of the failure to perform urine tests for sugar. The omission of insulin by many young diabetics is indicative of serious emotional or situational disturbances. Resentment against the irksome restraints imposed by the

disease or revolt against parental authority may lead to insulin omission. Repeated episodes of diabetic acidosis in young diabetics may be symptomatic of more serious psychic disturbances (20, 33, 35). In these patients insulin omission may have a feigned suicidal significance. An exploration of the situational and psychological factors responsible for the development of diabetic acidosis is an important phase in the care of cases after recovery from acidosis.

Impairment of insulin action often leads to diabetic ketoacidosis. Infection is the most important example of this condition. Activation of the adrenal cortex, fever, and general toxemia combine to impair the effectiveness of insulin action in infection. Surgical stress, acromegaly, hyperthyroidism, and pregnancy may also lead to diabetic acidosis by *increasing insulin requirements. Insulin resistance on an immunologic* basis may develop in certain diabetic patients and lead to diabetic acidosis (Chap. 21).

Differential Diagnosis

After a careful examination of the patient and the performance of a urinalysis, the diagnosis of diabetic acidosis is seldom in doubt. The possibility of insulin coma exists in all patients receiving insulin. Some of the features that distinguish this condition from diabetic coma are presented in Table 37-3 (also, see Chapter 48). Urine obtained from the bladders of patients in insulin coma may contain glucose and ketone bodies but rarely are the amounts such as to suggest diabetic acidosis.

Errors arise from the failure to consider diabetic acidosis (with subsequent delay in urinalysis) in patients with stupor, coma, abdominal pain, hyperventilation, and dehydration of undetermined origin. A number of disease states may mimic diabetic acidosis, leading to some diagnostic confusion. Severe hyperglycemia with blood sugar values in excess of 1,000 mg. per 100 ml. may develop in certain patients after a cerebral vascular accident. Although mild ketonuria may be present, ketonemia as detected by qualitative bedside procedures is always mild. Acidosis is generally nonexistent or mild. The hyperglycemia that occurs in an occasional case of meningitis may have a similar central nervous system mechanism.

Salicylate toxicity often presents with hyperpnea, and examination of the urine may give positive sugar reduction tests with mild ketonuria. Closer examination will resolve the diagnostic problem. Hyperglycemia is usually absent and specific enzymatic tests for urine glucose will give negative or slight reactions. Hyperventilation in salicylate toxicity leads to alkalosis in the early stage of the disease but acidosis may later supervene.

TABLE 37-3. DIFFERENTIAL DIAGNOSIS OF DIABETIC COMA AND INSULIN SHOCK

	Diabetic coma	*Insulin shock*
History	Diabetes may not have been recognized previously	Usually known diabetic receiving insulin
	Onset over a day or more of drowsiness, stupor, and finally coma	Onset rapid. May be preceded by excitement and confusion, "drunk." Headache common in reactions to P.Z.I.
	Nausea, anorexia, and vomiting common	Hunger
Physical examination	Respirations deep and rapid	Normal or shallow respirations
	Dehydration evident in skin turgor, mucous membranes, and ocular tension	Hydration normal
	Decreased reflexes. No sympathetic overactivity	Frequent twitching or spasms. Convulsions occur occasionally. Reflexes usually present. Sympathetic overactivity common (sweating, tachycardia and dilated pupils)
Laboratory		
Glycosuria	++++	0 (if obtained by catheter after emptying bladder)
Ketonuria	++++	±
Ketonemia (qualitative)	++	0
Therapeutic response to glucose given by vein	0	Some improvement evident promptly in most patients

Symptoms and Signs

The onset of diabetic acidosis is usually preceded by an intensification of diabetic symptoms. Polyuria increases and is accompanied in its early stages by an uncontrollable thirst. Gastrointestinal symptoms occur with regularity. Anorexia and nausea in many cases is followed by vomiting. These symptoms will lead an improperly educated diabetic patient to discontinue insulin because meals are missed. The importance of anorexia and vomiting in the pathogenesis of acidosis has already been discussed. Failure of water intake plus fluid loss resulting from vomiting accelerates dehydration. Abdominal pain occurs with frequency in children and young adults but rarely occurs in older dia-

betic patients. It has been suggested that dehydration may lead to a type of sterile peritoneal serositis. Pleural pain of a similar nature may be present in diabetic acidosis.

The clinical hallmarks of dehydration are always prominent. Conjunctival, oral, and nasopharyngeal mucous membranes are found to be dry and caked with inspissated secretions. The skin is dry and lacking normal turgor. The eyeballs appear sunken in the orbits and are soft on palpation.

Hypovolemia leads to signs of disturbed cardiovascular function, of which tachycardia and hypotension are prominent in severe cases. It is rare, however, to find the degree of peripheral vasoconstriction that is associated with traumatic shock.

The respiratory signs of diabetic acidosis have long been recognized. Hyperpnea becomes severe when there is a reduction of the blood pH below 7.2. Kussmaul respirations are notable in being rapid, deep, and without obvious subjective dyspnea. Cyanosis occurs only with profound circulatory collapse. Rales may not be heard in the lungs in the presence of pneumonia until rehydration has been achieved.

The systemic manifestations of diabetic acidosis include weakness, malaise, and prostration. Muscular aches and cramps may occur.

Depression of central nervous system function in diabetic acidosis begins with headache and drowsiness. Stupor and coma follow if the condition is untreated. A careful evaluation of the central nervous system function is of prognostic importance. The designation of coma should be reserved for patients who are unresponsive to painful stimuli. Lesser degrees of depression should be called semicoma or stupor. The reflexes and muscular tone are symmetrically depressed. The eyes roll aimlessly and the pupils are generally dilated and equal.

Clinical Laboratory Findings

Urinalysis will lead to the correct diagnosis of virtually all cases of diabetic acidosis by demonstrating severe glycosuria and ketonuria. The few exceptions can be attributed to profound depression of the rate of glomerular filtration and urine secretion. The sensitivity of the nitroprusside reaction for acetoacetate means that a strongly positive urine test can occur in the absence of significant ketonemia. Lee and Duncan have advocated using the same nitroprusside containing tablets or powders for serum as are used for urine testing. Four plus reactions of undiluted serum are seen only in diabetic acidosis and indicate that the serum ketone level is at least 40 to 50 mg. per 100 ml. Further dilutions of serum allow a rough estimate of the serum ketone level, which

has been of assistance in assaying prognosis and guiding therapy. It is unfortunate that rapid and accurate methods for measuring β-oxybutyric acids in the blood have not been developed. Values as high as 368 mg. per 100 cc. of plasma, expressed as acetone, have been found using quantitative chemical methods.

Chemical examination of the blood provides further substantiation of the diagnosis. Hyperglycemia between 400 and 800 mg. per 100 ml. is usually found. Blood sugar elevations as high as 2,000 mg. per 100 ml. have been observed. An occasional patient who has received large doses of insulin prior to hospitalization may have lower blood sugar levels in the presence of persisting acidosis.

The serum bicarbonate level, measured as the carbon dioxide combining power, is below 15 mEq. per liter in moderately severe acidosis. Values as low as 2 to 3 mEq. per liter may be present in profound acidosis. Although there is a general correlation between the clinical state and the degree of lowering of the bicarbonate level, striking individual exceptions are frequently encountered.

The determination of the hydrogen ion concentration of arterial or venous blood is becoming more practical at a clinical level. The rapidity and accuracy of the glass electrode method and the great physiologic significance of the determination of pH makes this an excellent way to assess the metabolic state of a patient and the response to treatment. Observed values in diabetic acidosis are presented in Figure 37-1.

Although large amounts of sodium and chloride ions are lost from the body during the development of diabetic acidosis, the concentration of these ions in the plasma of a patient with diabetic acidosis is the resultant of the separate losses of extracellular water and salt bal[illegible]

ser[illegible]

to[illegible]

Acidosis and the impairment of renal hemodynamics frequently lead to a mild hyperkalemia prior to therapy even with intracellular potassium depletion. A similar tendency is evident in the serum calcium and phosphorus levels although these serum components are rarely measured clinically.

A polymorphonuclear leukocytosis, between 15,000 and 30,000 cells per cubic millimeter, occurs in nearly all cases of diabetic acidosis. Even higher values have been reported in the absence of infection; consequently, the white blood count is useless in distinguishing infection from diabetic acidosis.

TREATMENT OF DIABETIC COMA

The aims of treatment in diabetic acidosis are: first, restoration of normal carbohydrate, fat, and protein metabolism by the administration of insulin; second, reconstitution of the extracellular and intracellular fluids by the administration of water and salts; third, prompt recognition and treatment of the circulatory and other complications.

General Measures

The care of a patient with diabetic coma requires a smoothly functioning team with the responsible physician always the captain. Preparations are started as soon as word reaches the floor of the expected arrival of the patient. Provision is made for house staff, nursing, and laboratory coverage. Insulin and fluids for intravenous administration in ample amounts should be available on the floor ready for immediate use.

Urine and blood specimens can often be obtained in the hospital receiving room so that laboratory examinations can be expedited. Physical examination includes a careful evaluation of the state of hydration, central nervous system, respiratory and cardiac function. Possible complicating infections are searched for. When peripheral veins are small and fragile, a polyethylene catheter is inserted into an antecubital vein. Gastric lavage is not mandatory unless signs of gastric dilatation are detected or recent vomiting has occurred. Late gastric atony is uncommon now that potassium salts are used in therapy and fluids are not taken by mouth until gastric emptying seems certain. Undue exposure of the patient is avoided and wool blankets *without* hot water bottles are provided.

A diabetic coma chart is instituted at the earliest possible moment in which the laboratory, clinical, and therapeutic data are recorded systematically. Urine sugar and ketone bodies are measured at hourly intervals until recovery. The minimal chemical examinations of the blood on entry include HCO_3, glucose, and urea or nonprotein nitrogen When available, blood pH and plasma sodium and potassium determinations are helpful. In comatous patients the attending physicians should not hesitate to repeat these parameters at intervals of 4 to 6 hours.

Continuous professional attention is essential and the comatous patient should never be left unattended by a physician until recovery is well established. Many complications of diabetic acidosis occur with great abruptness and require prompt countermeasures. Systematic observation of the blood pressure, pulse, respirations, mental state, and degree of hydration are carried out at one-half hour to hourly intervals

at first. The amount of insulin and the volume and nature of all parenteral fluids administered are tabulated chronologically. Only by careful recording of all this information can the attending physician achieve the proper perspective for intelligent therapy.

Insulin Administration

The absolute or relative lack of insulin is the basic deficiency of diabetic acidosis. Refractoriness to the action of insulin exists in nearly all patients with diabetic acidosis. Some of the insulin resistance can be attributed to the acidosis itself. Activation of the adrenal cortex, previously discussed, contributes to the unresponsive state. Serum from acidotic diabetic patients has also been demonstrated to contain a protein inhibitor of insulin that can be demonstrated in in vitro tests (16) (also *see* Chap. 20).

Considerable attention has been paid to the question of insulin dosage in diabetic acidosis. Many feel that large doses should be employed in the early hours of therapy. A comparative study has been conducted of three dosage schedules of insulin in a single clinic (38). It was found that the duration of coma and the time required for the blood sugar to reach 300 mg. per 100 cc. were the same in patients receiving 80, 160, or 240 units of insulin every 2 hours. Despite the results, there have been individual case reports in which truly massive doses of insulin seemed to be required before correction of diabetic acidosis was achieved. The question, therefore, is not the minimum insulin dose that will correct acidosis in a group of diabetic patients, but the optimum dose that will produce the most rapid correction of the metabolic disturbances and prevent undue delay in the recognition of the unusual patients requiring tremendous doses.

The author's recommendation is for an initial dose of 200 to 300 units of crystalline insulin in profoundly acidotic patients. Part of this insulin may be administered intravenously. This latter route is particularly important in patients with circulatory collapse when adequate absorption of insulin from the subcutaneous depots may be impaired. Insulin administration is continued with doses of 50 to 100 units per hour until a significant decrease in blood sugar or glycosuria becomes evident. This break in hyperglycemia indicates that the patient is not one of the relatively rare cases in whom truly massive doses of insulin are necessary. Insulin doses should be increased if no fall in blood sugar is evident after three to four hours of treatment. Once there is a fall of the blood sugar to 300 mg. per 100 cc. or a decrease of glycosuria to 3+, insulin doses should be reduced greatly to avoid hypoglycemia.

There is less urgency in the treatment of patients with mild acidosis

or simple ketosis and much smaller doses of insulin are required. Insulin resistance may be mild in these cases and hypoglycemia is a real hazard with the larger doses of insulin described above.

Fluid and Electrolyte Repletion

It is helpful to consider the replacement of fluids and electrolytes in diabetic acidosis in two phases. During the initial phase lasting 3 to 4 hours, the primary objective is the restoration of the extracellular fluid volume because of the great importance of regaining normal cardiovascular function. Provision of extra water and intracellular electrolytes is provided in the second phase of fluid therapy.

Extracellular Fluid Restoration

The choice of fluids for expansion of the extracellular space requires some comment. Undoubtedly acidosis itself is directly harmful to the organism. The muscular work of hyperventilation is considerable; intracellular electrolyte loss is promoted; peripheral vascular resistance is decreased; and cerebral function may be adversely affected by acidosis. For these reasons, it is desirable to lower the hydrogen ion concentration without delay. Sodium lactate, ⅙ molar, is well suited for this task because it usually is available for immediate use, whereas sodium bicarbonate requires preliminary dilution in distilled water. The use of molar sodium lactate in the treatment of diabetic acidosis is contraindicated. This solution is strongly hypertonic and will accentuate intracellular dehydration, unduly increasing extracellular fluid osmolarity. The aim of alkali therapy is not to restore the serum bicarbonate to normal but to allay severe acidosis. Formulas for calculating lactate administration are not based on sound physiological premises and the best guide to therapy is the response of the patient. An evident decrease in the respiratory effort is usually achieved with rapid administration intravenously of one or at most two liters of ⅙ molar sodium lactate. The use of larger amounts of sodium lactate is liable to induce a significant alkalosis at a later stage in treatment. The plasma bicarbonate may be an unreliable index of the hydrogen ion concentration in diabetic acidosis. It has been observed that hyperventilation with a low pCO_2 tends to persist despite a rising plasma bicarbonate and by this mechanism a mild respiratory alkalosis may succeed a severe metabolic acidosis. It has been suggested that the lag in the recovery of spinal fluid bicarbonate concentration may be responsible for the persisting hyperventilation (42).

The sodium lactate solution is followed by Ringer's solution containing lactate in the proposed therapeutic scheme. This solution closely

resembles extracellular fluid in its electrolyte composition and is ideally suited for extracellular fluid restoration. It is definitely preferable to 0.9 per cent sodium chloride because *it contains chloride at a concentration that approximates the normal concentration of chloride in the extracellular fluid.* Isotonic saline solution, on the other hand, contains an excessive concentration of chloride; when it is given in large amounts, metabolic acidosis may be aggravated by hyperchloremia (32, 37). Usually a total of 3 liters of fluids of both types is required during the first phase of treatment in an adult patient. Substantial clinical improvement should be apparent after this phase of treatment. In most cases hypotension will be corrected and the circulation greatly improved. Acidosis will be decreased and a fall in blood sugar evident.

Provision of Water and Intracellular Electrolytes

During the second phase of treatment there is a greater need for free water, for intracellular electrolytes, and for carbohydrate. Excellent special electrolyte mixtures have been devised by Butler (9) and by Naborro and associates (31) (see Table 37-4). Because these mixtures

TABLE 37-4. POLYELECTROLYTE MAINTENANCE SOLUTIONS ADVOCATED FOR THE TREATMENT OF DIABETIC ACIDOSIS

Author	Na^+	K^+	Mg^+	Ca^+	Cl^-	HCO_3^- *(or lactate)*	*Phosphate*	*Carbohydrate*
	mEq/Liter							*G/Liter*
Butler (9)	42	30	5	—	35	26	16	75 glucose
Nabarro (31)	20	30	5	—	45	—	10	50 glucose
Harwood (18)	30	16	—	—	24	20	3	50–100 glucose
Martin (26)	57	21	6		50	25	21	0 with [illegible]0 (fructose)

may not be readily available in most hospitals it is convenient to use mixtures of simpler parenteral fluids. Mixing Ringer's solution containing lactate with a 5 or 10 per cent sugar solution either directly or by administration through the same needle provides free water for intracellular rehydration and continuing urinary loss. Very significant hy-

pernatremia and hyperchloremia occurs frequently when only isotonic sodium chloride is used in treatment. This mixture is administered more slowly than the solutions used heretofore.

Plasma potassium levels are generally moderately elevated before therapy is started. A fall, frequently to hypokalemia levels, occurs during the first 4 to 8 hours of treatment. This fall can be attributed to continued urinary loss, dilution of the extracellular fluids with potassium free solutions when these are used, and re-entry of potassium into the cells. It has been shown (8) that a high extracellular fluid hydrogen ion concentration (low pH) is associated with a rise in plasma potassium in a number of situations even without significant alterations in total body potassium. The converse holds true in alkalosis. The acute changes in plasma potassium level in diabetic acidosis may be determined to a considerable extent by the plasma pH. If the urinary output is good and the response to insulin has been satisfactory it is unnecessary to wait for clinical and electrocardiographic signs of potassium deficiency before including potassium in the administered fluids. A determination of the plasma potassium concentration is helpful in patients still in severe acidosis and responding slowly to treatment.

Potassium phosphate has an advantage over the usually employed chloride salt, because it furnishes phosphate that has been lost from the intracellular fluid rather than chloride, which is already adequately provided. Forty milliequivalents of the buffered potassium phosphate may be safely added per liter of fluid when cautiously administered intravenously over 2 to 3 hours. In exceptional cases higher concentrations of potassium may be required in hypokalemic patients.

The total amount of potassium that may be given during the first 12 hours of treatment is highly variable and depends upon the degree of pre-existing depletion, rapidity of recovery from acidosis, and the rate at which glycogen stores are replenished. Between 100 and 200 mEq of potassium will usually maintain normal extracellular fluid potassium levels until potassium-containing nutrients (e.g., orange juice or beef broth) can be taken by mouth.

The electrocardiogram gives important information concerning the level of potassium Initially the tall, peaked T waves and other changes of hyperkalemia may be present. Later prolongation of Q-T interval, depression of the ST segment, low and inverted P waves, depressed or diphasic T waves, and prominent U waves may be found suggesting hypokalemia. Caution should be exercised in the use of this fast and available diagnostic aid. The electrical abnormalities recorded in the electrocardiogram are not determined solely by the plasma potassium level but are profoundly altered by intracellular potassium concentra-

tion, the other electrolyte abnormalities, and the complications introduced by primary cardiac disease and drugs acting on the heart. In one series of 42 abnormal electrocardiograms in diabetic acidosis no correlation could be established between the electrocardiographic changes and the serum potassium level in 21 (19). Frequent determinations of plasma potassium should not be neglected in patients who seem to require large amounts of potassium or whose progress is in any way unsatisfactory.

Carbohydrate Supplementation

Carbohydrate is given to the patient intravenously after the first 3 to 4 hours as a vehicle for free water and to promote restoration of carbohydrate metabolism. Ten years ago the use of glucose was a topic of violent controversy. Now most students of diabetic acidosis advocate the use of sugar after the blood sugar concentration has fallen significantly. The fact that fructose disappears from the blood at a normal rate in diabetes has been cited as a reason for utilizing this sugar in preference to glucose in the treatment of diabetic acidosis. Although it is true that fructose uptake by tissues is not dependent on insulin, considerable conversion of fructose to glucose in liver occurs in diabetes, reducing the potential advantage of fructose over glucose.

The degree to which glucose or fructose accelerates recovery from ketonemia has been examined under controlled conditions by Rosecan and Daughaday. Diabetic patients were allowed to become moderately ketotic under careful hospital supervision. Regular insulin was then given in doses adequate to control hyperglycemia during the administration of 0.9 per cent sodium chloride over a 4-hour period. At a later time ketosis was reinduced in the patient and either glucose or fructose was administered to the same patient receiving the dose of insulin used previously. A total of 20 episodes of ketosis were studied in 8 diabetic patients, results of the different therapies were directly compared in individual patients. The rate of fall of the blood ketones was measured and was found to be significantly faster in the presence of either glucose or fructose than with saline solution (Fig. 37-3). This observation indicates that, even in the presence of hyperglycemia, additional carbohydrate accelerated recovery from ketosis. It was impossible to detect a significant advantage of fructose over glucose in respect to recovery from ketonemia but with fructose the fall in blood sugar was more prompt.

Delay in carbohydrate therapy is advisable to avoid intensifying hyperglycemia with resultant extracellular fluid hyperosmolality, and osmotic diuresis. After insulin action has commenced the use of fructose

or glucose solutions in the suggested concentrations does not significantly delay the fall of the blood sugar.

At a practical level the differences between glucose and fructose in the treatment of diabetic acidosis are not large. Fructose has the advantage of being more readily utilized by the liver in diabetes but

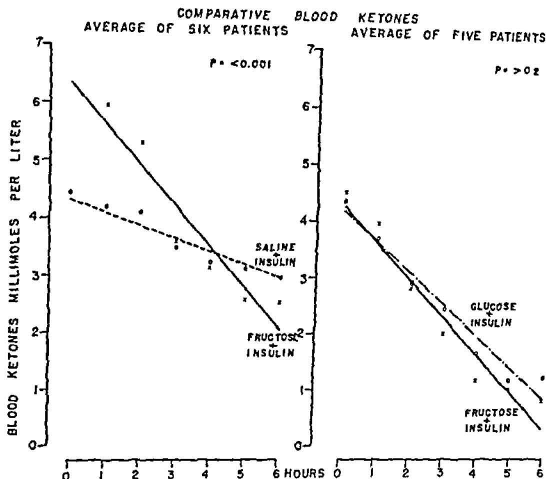


FIG. 37-3. Recovery from ketonemia by diabetic patients treated with insulin and either saline solution or 10 per cent sugar (fructose or glucose). In this clinical study the addition of carbohydrate accelerated recovery in the insulin treated patient. (*From* Rosecan, M., and Daughaday, Wm. H.: *J. Clin. Invest* 33:49, 1954.)

much of the fructose is transformed by the liver into glucose and reenters the circulation. Nonetheless, blood sugar levels return to normal faster with fructose than with glucose. One possible disadvantage of fructose in the later stages of treatment is the poor utilization of this sugar by the central nervous system. Hypoglycemia can occur at the

same time that reducing sugar is present in the urine, owing to continued fructosuria. Urinalyses with enzyme (glucose oxidase) impregnated paper provide more reliable guides for treatment at this stage.

Complications

Fortunately the response to treatment is uneventful and recovery occurs promptly in the vast majority of patients with diabetic acidosis. A small fraction of the cases presents real therapeutic problems such as major complicating infection or other disease states. The development of symptomatic hypokalemia is now rare because of the incorporation of potassium into the replacement fluids. An ascending muscular paralysis with eventual involvement of the respiratory muscles is the most dramatic and dangerous of the manifestations of hypokalemia. Disturbed cardiac function with a susceptibility to digitalis toxicity is another important hazard of hypokalemia. Gastric atony and intestinal ileus, formerly a common finding in severe diabetic acidosis, now is occurring much less often when potassium is used in therapy. The mild alkalosis that commonly was seen late in the recovery stage after treatment with sodium lactate can be largely eliminated by potassium treatment permitting the renal excretion of excess bicarbonate.

Hypoglycemia is a complication of therapy that should be avoidable. With the large amounts of insulin used early in treatment the physician must be on the alert for hypoglycemia after insulin responsiveness is regained. Prompt reduction of insulin doses and the use of parenteral and oral carbohydrate should be instituted when evidence of a return of the blood sugar toward normal is recognized.

The circulatory complications of diabetic ketoacidosis are responsible for most of the therapeutic failures. Hypotension occurs in many patients who enter the hospital in profound acidosis. Usually the blood pressure rises promptly after the administration of salt solutions. The administration of 6 per cent dextran has been advocated in the presence of hypotension. If anemia can be demonstrated, transfusion with whole blood is indicated. Occasionally the blood pressure does not respond to hydration, in others hypotension may first appear many hours after treatment has been instituted and lead to death. Case reports of such unexpected deaths have emphasized that they took place after biochemical improvement.

The mechanism of shock that is refractory to rehydration has been clarified by the studies of Howarth and her associates. Cardiac catheterization of five patients with refractory hypotension complicating diabetic acidosis was carried out. Right auricular pressures and cardiac

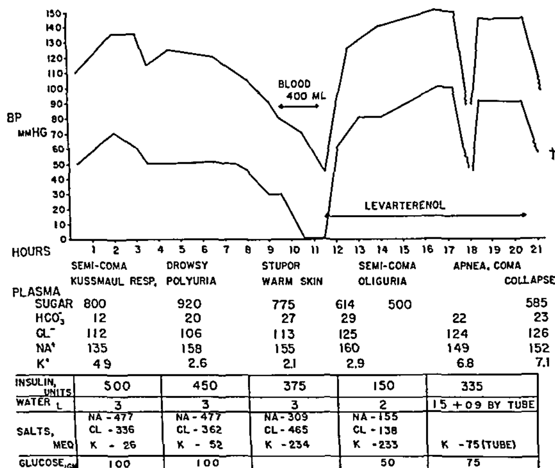
outputs were both found to be normal or slightly elevated, whereas in simple hypovolemic shock these parameters are both depressed. In view of the normal cardiac output, hypotension can only be attributed to decreased peripheral resistance that was estimated to be only half of normal. Normal vasoconstriction in the face of hypotension was therefore absent.

Few measurements of the circulation in different parts of the vascular bed in diabetic acidosis have been reported. Reduced blood flow in the hand, as measured by the plethysmograph, was found in acidotic patients with unresponsive hypotension by Schecter, Wiesel, and Cohn. Kety and co-workers, in their classic studies of the mechanism of disturbed central nervous system function in diabetic coma, found increased cerebral blood flow in comatose patients with, however, decreased oxygen uptake. The increase in cerebral blood flow was attributed solely to acidosis. Renal blood flow and glomerular filtration are both decreased in diabetic acidosis.

The factors that provoke late vasomotor collapse are poorly understood. Unrecognized hypokalemia may be a major factor but is absent in some cases. A critical review of the charts of patients with vasomotor collapse at Barnes Hospital suggests that the clinical response to treatment by these patients was usually delayed and characterized by uncontrolled hyperglycemia. Two cases treated on a general medical service have been selected to illustrate the problem of late vasomotor collapse.

The first patient (Fig. 37-4), a forty-six-year-old obese colored woman, entered the hospital in a semicomatose condition. Her acidosis was not profound as indicated by a plasma carbon dioxide combining power of 12 mEq per liter Her response to 500 units of insulin administered during the first 4 hours of treatment was not satisfactory in that the blood sugar actually rose from 800 mg. per 100 ml. on entry to 920 mg. per 100 ml. Some of this rise may be attributed to the injudicious use of glucose, which contributed to the hyperglycemia. Despite the fact that the skin continued to be warm, the radial pulse strong, and polyuria persistent, there was a progressive fall in blood pressure, eventually leading to complete vasomotor collapse. Blood transfusion was without benefit but levarterenol, when finally used, did restore blood pressure to normal levels. Cerebral function, however, remained poor with weak, shallow respirations Cardiac arrhythmias developed and eventually led to death. Hypokalemia, which developed early, may have contributed to the collapse. Later moderate hyperkalemia occurred despite the fact that careful monitoring with the electrocardiogram failed to show the expected changes of hyperkalemia.

The second case illustrates the fact that vasomotor collapse may occur late in the course of treatment. The patient (Fig. 37-5), a sixteen-year-old girl, entered the hospital in severe diabetic acidosis with a plasma bicarbonate combining power of 7.4 mEq. per liter. As she was only drowsy her condition was not considered alarming and she received



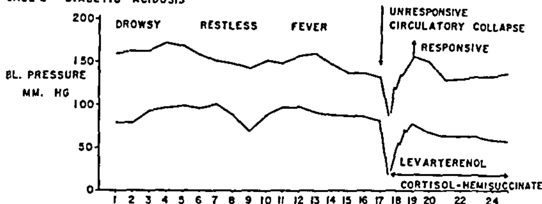
PLASMA							
SUGAR	800	920	775	614	500		585
HCO_3^-	12	20	27	29		22	23
CL^-	112	106	113	125		124	126
NA^+	135	158	155	160		149	152
K^+	4.9	2.6	2.1	2.9		6.8	7.1

INSULIN, UNITS	500	450	375	150	335
WATER L	3	3	3	2	1.5 + 0.9 BY TUBE
SALTS, MEQ	NA - 477 CL - 336 K - 26	NA - 477 CL - 362 K - 52	NA - 309 CL - 465 K - 234	NA - 155 CL - 138 K - 233	K - 75 (TUBE)
GLUCOSE, GM	100	100		50	75

Fig. 37-4. The hospital course of an example of delayed vasomotor collapse with eventual death in diabetic acidosis is summarized The patient, a 46-year-old obese woman, had experienced diabetic symptoms for three weeks. Vomiting and prostration had occurred on the day prior to entry. Deep breathing and severe lethargy prompted hospitalization. (*From* Daughaday, Wm. H.: *Diabetes* 7.230, 1958.)

only physiologic saline solution and modest doses of insulin during the initial phase of treatment. She did not respond adequately to treatment in that the plasma bicarbonate combining power had risen only to 7 7 mEq after 8 hours of treatment. Fever without ascribable cause, restlessness, and mental obtundity developed, suggesting disturbed

hypothalamic function. Vasomotor collapse occurred precipitously 17 hours after entry and was immediately recognized by the attending physicians. The benefit achieved with levarterenol was unequivocal but eventual recovery occurred slowly with gradual subsidence of the central fever and the re-establishment of vasomotor control.



BL. SUGAR, MG % 425 363 382

HCO_3^-, MEQ/L 74 7.7 8.1 17.2

NA 150
CL 125
K 3

INSULIN, UNITS	140	120	20	20	10	
WATER, LITERS	35	2	1.7	1.3	1.7	1.4
ELECTROLYTES MEQ	NA 542 CL 542 K	NA 138 CL 116 K 5	NA 100 CL 26 K 26	NA 50 CL 59 K 59	K 40	K 40
GLUCOSE, GM	0	50	78	68	88	70

FIG. 37-5. The hospital course of an example of delayed vasomotor collapse with recovery after levarterenol treatment. Diabetic symptoms had been noted by this 16-year-old girl for one month. Weakness had progressed for one week before entry. Obtundity and deep breathing which had been present for one day prompted hospitalization. (*From* Daughaday, Wm. H.: *Diabetes 7:* 230, 1958.)

Several aspects of this complication deserve emphasis:

1. Peripheral vasomotor collapse may be present on entry or develop during the course of treatment of diabetic acidosis.

2. Cutaneous signs of vasoconstriction may not occur or appear late and delay clinical recognition of the circulatory disorder.

3. Blood pressure determinations, carefully obtained at frequent intervals and faithfully recorded, permit early recognition of vasomotor instability.

4. Blood transfusions are of little benefit if hypovolemia has been corrected and may precipitate pulmonary edema. Potent vasoconstrictor drugs such as levarterenol, when used promptly, are of real value.

Despite the frequency of hypotension, hemoconcentration, and profound dehydration it is remarkable that serious renal tubular damage is not a common complication of diabetic acidosis. In a review of 476 adult patients with diabetic acidosis who had been hospitalized one or more times, only three exhibited evidence of progressive azotemia during therapy (40). The maximal azotemia in these patients was reached on the fourth day with subsequent gradual improvement. Severe oliguria was not evident in any of the three patients but has been observed in one patient at Barnes Hospital. In explaining the low frequency of serious renal damage in diabetic acidosis the authors suggested that the period of shock and resulting renal ischemia was usually not long enough to produce the overt manifestations of renal tubular damage. An alternative explanation would be that renal vasoconstriction in the presence of diabetic acidosis may not be as great as in other conditions more commonly associated with tubular damage. Evidence for impaired vasoconstriction in the vascular beds has been discussed already.

Therapeutic Response

With modern therapy most patients exhibit progressive improvement. The blood sugar falls below 300 mg. per 100 cc. by 6 hours in the average patient and nearly all patients achieve this state by 12 hours. Ketonuria generally persists for 8 to 12 hours when measured with the sensitive nitroprusside reaction. The recovery of normal extracellular fluid volume and intracellular water have been found to occur within 24 to 48 hours in balance studies. The complete reconstitution of the intracellular electrolytes may take as long as 10 days (Fig. 37-6) and regaining of lost protoplasm (nitrogen balance) is even more protracted (29). The recovery of the carbohydrate tolerance and insulin requirement existing prior to acidosis may similarly be delayed.

Improvement in central nervous system function occurs rapidly in young patients but in older diabetic patients, presumably suffering from some cerebral arteriosclerosis, coma may last for 24 hours or even longer and be followed by delirium and confusion for several days.

The long-term detrimental effects of diabetic coma are less well known. Pregnancy seems to be adversely affected and many have suggested that episodes of diabetic acidosis accelerate the appearance of diabetic degenerative vascular disease.

The recovery period after diabetic acidosis presents special problems in diabetic regulation, owing to the irregular return to normal food intake and changing insulin requirements. In diabetic patients whose insulin requirements prior to acidosis are well known it is safe to resume

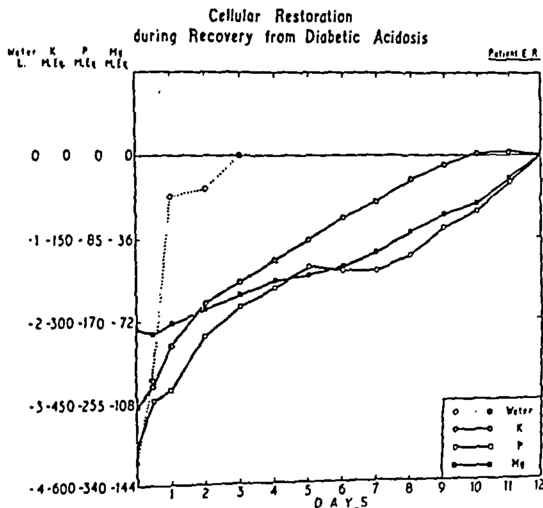


FIG. 37-6. The delayed recovery of cellular constituents is presented in this graph showing the cumulative cell balances of water and electrolytes corrected for changes in cell nitrogen. Ordinate scales in proportion to normal concentrations of potassium, magnesium and labile phosphorus in cell water (From Nabarro, J. D. N., Spencer, A. D, and Stowers, J. M. *Quart. J. Med.*, *21*:225, 1952)

intermediate insulins (isophane or lente) in full or two thirds of the original dose as soon as normal eating has been regained. Usually this insulin dose will need supplementation for a number of days with crystalline insulin on the basis of premeal urinalyses for sugar. Treat-

ment with intermediate action insulins might be started on the second day after diabetic acidosis for previously unrecognized diabetic patients. Initial doses of 20 to 30 units are supplemented with crystalline insulin. Later adjustments are made, depending on the response.

Prognosis

The failure of a patient to improve under therapy should lead to a careful re-evaluation of the metabolic state and a search for complicating conditions. Of the severe occult complicating infections, fulminant pyelonephritis with papillary necrosis deserves mention. Also, the diagnosis of acute pancreatitis complicating diabetic coma is also frequently missed. This disease is more common in diabetics than nondiabetics and when it leads to diabetic acidosis the prognosis is particularly bad. In one series of five patients, four succumbed (6).

Many hospitals and clinics have reported their results in the treatment of diabetic acidosis. In recent series the mortality has ranged from 30 per cent to 1.5 per cent. To some extent the wide differences in results represent the criteria used for inclusion of cases and the relative severity of the cases treated. The magnitude of the therapeutic problem is determined primarily by the degree and duration of the central nervous system dysfunction, the adequacy of the circulation, and the age of the patient. Other prognostic features include the presence of complicating illnesses and the degree of biochemical abnormality. In general, municipal hospitals and other institutions serving indigent patients receive a higher percentage of cases with a graver risk. Some of the increased mortality from such institutions can be attributed to this fact. The therapeutic skill of a smoothly running team should not be underestimated, however. The experience at the Massachusetts General Hospital is a case in point (18). An intensive effort to improve professional care, to obtain more active consultation with the visiting staff, and to provide better laboratory coverage was combined with the introduction of the use of more physiologic parenteral fluids. With these improvements the mortality rate dropped from 20 per cent to 1.5 per cent. The last 54 patients were treated without a death.

A certain number of deaths undoubtedly cannot be prevented. Two of the last 5 patients who died of diabetic coma at Barnes Hospital were over the age of 80 and died soon after entry to the hospital. A third patient had profound arteriosclerosis with bilateral amputations and old and recent myocardial infarction.

The current low mortality observed in the treatment of diabetic acidosis in the better clinics makes difficult on the basis of survival the comparison of different therapeutic regimens. While this is the ultimate

test, further refinements in therapy will be developed by comparing the promptness and smoothness of the reversal of the metabolic abnormalities.

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Chapter 38

CARDIOVASCULAR DISEASE IN DIABETES

Henry T. Ricketts

Whereas some 30 per cent of all deaths in the United States are due to vascular disease, among persons with diabetes this figure has reached 70 per cent. The high incidence in the latter group, while to some extent it relates to age, is most clearly associated with the duration of diabetes even in younger patients. Thus, White and Waskow have reported that of 200 such patients whose primary disease had begun in childhood and who had survived for 20 years or more, 92 per cent had vascular lesions at the time of examination. Although many of the group had retinopathy and nephropathy, which are primarily of capillary origin, 75 per cent showed the lesions of the arterial system with which we are concerned in this chapter.

ETIOLOGY

It is all very well to say that the occurrence of arteriosclerosis is related to the duration of diabetes, but we are still uncertain as to what element of diabetes, operating over many years, is responsible. Most authorities agree that, despite some important exceptions, poor control of the diabetic state is associated with a higher and good control with

a lower frequency of vascular complications of all kinds. The outstanding biochemical features of poorly controlled diabetes are hyperglycemia and hyperlipemia. If the fate of the vessels is determined by the quality of the blood that bathes them (and this is not fully established), then it is to the sugar and lipids of the blood that we might logically direct our attention.

The possible relationship of glucose metabolism to vascular disease by way of the mucopolysaccharides has been discussed in Chapter 13. It is too new an area to justify any but a speculative clinical application. In contrast, the field of lipid metabolism in relation to atherosclerosis has been explored with increasing intensity for years—one is tempted to say to the point of exhaustion.

The evidence that, in the general population, there is some association between high intake of animal fat, elevated levels of serum lipids, and the occurrence of atherosclerosis, as manifested chiefly by coronary disease, has reached impressive proportions. Such association, however, is not sufficiently constant to be of predictive value in individual cases, and the exceptions and inconsistencies are such as to force the conclusion that hyperlipemia alone cannot account for all the observed facts.

As with nondiabetics, so also with diabetics. The latter, to be sure, tend to have somewhat higher lipid levels than those of normal persons of comparable ages; but in the long-term, treated patient, who after all is the one with whom we are really concerned, these differences are surprisingly small* and seem inadequate to account for the more than twofold increase in the percentage of deaths from vascular causes among diabetics as compared with the population at large. Furthermore, there is but a poor correlation between concentrations of various circulating lipids and the presence or absence of demonstrable arteriosclerosis. Pomeranze and Kunkel, for example, in a study of 273 diabetic patients found that, of those with total serum lipids above 750 mg. per 100 ml., 78 per cent had severe atherosclerosis while 22 per cent had a moderate amount or none, however, of those with normal values, 40 per cent had severe lesions and 60 per cent had lesions of moderate severity or none. Comparable studies in which cholesterol and lipoproteins were measured have shown similar correlations. These remarks must not be construed as representing a nihilistic view of the fat theory. They are intended only to point out that fat concentration per se does not explain all the observations.

* Of the order of 30 to 40 mg. per 100 ml in persons under 40 years of age, with no significant difference in those over 40, according to Lundbaek.

The indisputable fact that some patients with early and obviously mild diabetes have severe arterial disease while others with poorly controlled glycosuria of many years' duration seem to have escaped raises the question of whether in certain individuals the tendency to an inferior vascular system is genetically transmitted along with the tendency to diabetes. While this may be true, it is difficult to prove. Apparently diabetes per se, induced in patients with a negative family history by generalized disease of the pancreas or by its surgical removal, can result in typical diabetic retinopathy and premature atherosclerosis. Cases of this kind have been reported separately by Lawrence, Sprague, and Burton, *et al.*, and while hereditary influences in these instances cannot be completely excluded, they do seem improbable.

PREVENTION

The higher prevalence of vascular disease among the obese emphasizes the need for weight reduction in such patients.

Although the mechanisms by which diabetes predisposes to atherosclerosis are not precisely known, there is good reason to believe that

TABLE 38-1. DIFFERENCES IN COMPOSITION OF ISOCALORIC DIETS WITH HIGH AND LOW FAT

	42% of total calories as fat		*20% of total calories as fat*	
	Gm.	*Cal.*	*Gm.*	*Cal.*
Carbohydrate	200	800	345	1380
Protein	75	300	75	300
Fat	111	999	47	423
Total		2099		2103

hyperglycemia or some associated feature is important. If blood lipids play any role, it is well to remember that in the presence of excessive glycosuria, even without ketosis, both postabsorptive and postprandial levels of certain lipids are abnormally high and that they can be lowered with adequate amounts of insulin. Perhaps this is reason enough, although there are others, for careful regulation of the blood sugar.

Hypercholesterolemia can be minimized by diets low in fat. Severe restriction, however, is both unpalatable and, for the diabetic, impractical. The average American derives 40 to 45 per cent of his total

calories from fat. He could, if necessary, halve this figure and increase carbohydrate without incurring any physiologic penalty. But if, for example, in a 2100 caloric diabetic diet the fat calories were reduced to 20 per cent of the total, rearrangement of Table 38-1 would result as shown. The high carbohydrate content of the second diet increases the difficulty of diabetic control, and this in itself may raise blood lipid concentrations. If it were actually known which cholesterol levels are atherogenic and which are not, fat intake could be adjusted accordingly, even at some sacrifice in other areas. In the absence of such knowledge, drastic curtailment is not warranted. A reasonable compromise would be a diet in which the nonprotein calories are supplied about equally from carbohydrate and fat (Table 38-2).

TABLE 38-2. COMPOSITION OF DIET WITH MODERATE FAT CONTENT: NONPROTEIN CALORIES SUPPLIED EQUALLY FROM CARBOHYDRATE AND FAT

	Grams	*Calories*
Carbohydrate	225	900
Protein	75	300
Fat	100	900
Total		*2100*

It has been shown that the addition to the diet of vegetable fats containing essential or polyunsaturated fatty acids (linoleic, linolenic, arachidonic) significantly lowers serum cholesterol. Substitution of these for animal or saturated fats produces further lowering. The mechanism by which this occurs is unknown Artificially hydrated vegetable fats, it should be noted, have little such effect. In practice, complete replacement of animal with vegetable fats is not feasible because of unpalatability, and their simple addition would be unwise because the amounts required would lead eventually to obesity. Partial replacement, however, can be accomplished by the use of safflower, corn, or soya oil in cooking and salad dressings. Whether such measures will be widely accepted by patients remains to be seen, but they may be worth trying.

The finding that the administration of estrogen alters the lipoprotein pattern of men with atherosclerosis to resemble the pattern of premenopausal women, who are relatively immune, is of great interest It is doubtful, however, that many men would want to submit to this sort of prophylaxis over a period of years. Diabetic women, who seem to

lose the immunity of their sex with the advent of diabetes, are equally as good candidates for such treatment, but lack of real proof of its efficacy and the possibility of complications such as vaginal bleeding and uncomfortable engorgement of the breasts render its general application unwise and its acceptance unlikely at present.

HEART DISEASE

Nearly all heart disease in persons with diabetes is the result of coronary atherosclerosis. Contrary to the situation in the general population, diabetic women are as susceptible as diabetic men. Slowly progressive narrowing of the lumen may lead to myocardial ischemia with ensuing angina or fibrosis and gradual decompensation, while sudden occlusion is generally followed by infarction. The element of hypertension, when present, may be added to that of coronary sclerosis.

The clinical manifestations and treatment of coronary narrowing in the diabetic do not differ from those in the nondiabetic individual. These subjects are covered in standard works on cardiology so that only their special aspects will be discussed in this place.

Angina or Congestive Heart Failure

The occurrence of *angina or congestive failure* in the obese patient urgently demands reduction of weight. Such a patient who does not need insulin may be given a caloric allowance 25 to 30 per cent below that estimated for maintenance. Since cardiac disorders of any serious degree require some restriction of activity, this must be taken into account in the calculations. Here, the physician who worries about the evils of fat may relax, for a proper reducing regimen cannot provide much of it. Restriction of salt, when necessary, is as easily accomplished in a diabetic or reducing diet as it is in any other. For the obese patient who uses insulin, the institution of a weight-losing program should be accompanied by a reduction in insulin dosage of 25 or 30 per cent. Many such patients will be able to discontinue insulin entirely as body weight approaches normal.

Despite some opinion to the contrary, the concensus is that hypoglycemia may precipitate frank occlusion in patients with coronary disease and is therefore to be strictly avoided. While such accidents are rare and even the induction of anginal attacks is in the author's experience uncommon, the epinephrine response to hypoglycemia, with its elevation of blood pressure and tachycardia, is undesirable. For these reasons the blood sugar should be carried at somewhat higher levels in such patients than is generally considered ideal: say, above 150 mg.

per 100 milliliters. Coronary heart disease, however, is no excuse for permitting outspoken glycosuria or for withholding insulin when it is clearly needed.

Acute Myocardial Infarction

Acute myocardial infarction of relatively minor severity may occur in diabetic patients, as in others, with no cardiac symptoms. The event may be betrayed by an otherwise unexplained increase in glycosuria.

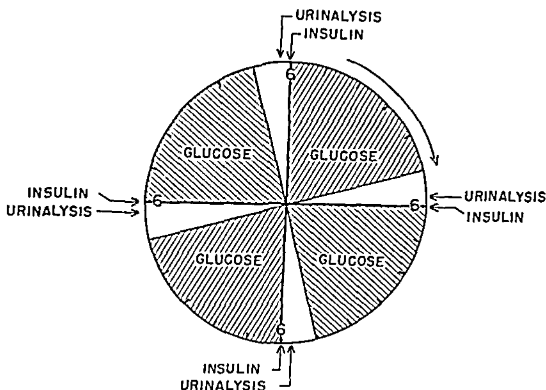


FIG. 38-1. Schema of diabetic management in emergencies.

Treatment of serious infarction is complicated by the presence of diabetes. The accident itself leads quickly to exacerbation of glycosuria and, in patients with severe diabetes, even to ketosis, sometimes requiring a sharp increase in insulin dosage Anorexia, nausea, and vomiting may prevent the intake of food and necessitate the administration of glucose intravenously. This raises the dilemma of providing as much glucose as is feasible for meeting nutritional needs and yet avoiding an excess of fluid that might further embarrass an already compromised heart.

These problems are best met by the application of Woodyatt's 6-hour

program (Fig. 38-1). Every 6 hours around the clock, 3 things are done in the following order:

1. The urine is tested for sugar by one of the qualitative copper reduction methods, not by strips or sticks, and, if the test is strongly positive, for ketone bodies. An indwelling catheter may be necessary, and if so, antibiotics should be employed prophylactically. The blood sugar should be determined at least once daily.

2. Soluble (regular or crystalline) insulin is given subcutaneously, *not* into the intravenous fluid container or tubing, in an amount determined by the urine test. Because of wide individual variations it is impossible to give a dosage formula that is valid for all cases. For the patient who has not previously required insulin, the following will serve as an illustration:

Urine sugar	0	+	++	+++	++++
Insulin (units)	0	0	4	8	12

For the patient who has previously taken insulin, it is assumed that for practical purposes the expected reduction in requirement owing to decreased food intake will be at least compensated and probably exceeded by an increased requirement owing to the "stress" response to infarction. Consequently, one fourth of the customary total daily dose is given as soluble insulin every 6 hours for a sugar-free or a + test, and to this dose extra amounts are added when glycosuria is greater. Thus, for the patient who has usually required 40 units of NPH insulin daily, the long-acting insulin is discontinued and 10 units of soluble insulin is given every 6 hours as the basic dose, with increments according to the urine tests. For example:

Urine sugar	0	+	++	+++	++++
Insulin (units)	10	10	10 + 4	10 + 8	10 + 12

The amount of additional insulin given at each period varies with circumstances and must be determined by trial and error. The aim should be to maintain slight to moderate glycosuria.

3. Five hundred milliliters of 10 per cent glucose in water is given intravenously at a rate of 100 ml. per hour. This supplies 2,000 ml. of fluid and 200 gm. of glucose (800 calories) per twenty-four hours. Each infusion is completed an hour before the beginning of the next period, thus allowing some time for re-establishment of fluid and glucose equilibrium Water in these amounts and at these rates is well tolerated even by very ill patients. Ordinarily, saline should not be used. If the patient cannot take nourishment by mouth for more than a day or two, 2 gm. of potassium chloride should be added to every liter of parenteral

fluid, provided renal function is good. In myocardial infarction, of course, the electrocardiogram cannot be relied upon to detect abnormal concentrations of potassium; chemical methods must be employed.

As the patient becomes able to tolerate oral feedings, these should be given in liquid to soft form at 3-hour intervals and in amounts sufficient to furnish 150 to 200 gm. of carbohydrate daily. Soluble insulin, however, should be continued at 6-hour intervals for several days. Long-acting preparations may be resumed when recovery is well advanced

Concerning the use of anticoagulants, opinion is still divided. At the University of Chicago Clinics they are employed almost routinely in diabetic as well as in other patients. Treatment is begun immediately with heparin and dicumarol, the former being given for one or two days only and the latter being continued in doses (50 to 100 mg. daily) sufficient to prolong the prothrombin time from 2 to 2.5 times normal. Therapy is maintained for at least 6 weeks and often permanently. Early fears that such treatment might lead to retinal hemorrhage in cases with retinopathy or to cerebral hemorrhage in the presence of hypertension and generalized arteriosclerosis have not been substantiated. Moreover, with a single exception, such accidents have not occurred in postinfarction patients being treated prophylactically for many months, even in the face of occasional hemorrhage into the gastrointestinal tract, kidneys and subcutaneous tissues.

Prevention

The prevention of coronary disease is the prevention of atherosclerosis. The avoidance of infarction in patients known to have coronary sclerosis is as difficult as it is desirable. Although hypertensive patients are more prone to such accidents than are other patients, there is no evidence that hypertension is immediately causative, and indeed attempts to lower blood pressure may provoke rather than prevent thrombosis. The finding that hyperlipemia favors clotting is another argument for low fat diets. In patients with progressively severe angina, serious thought should be given to the prophylactic use of anticoagulants. The usual measures of rest and the avoidance of nervous tension and heavy exertion should, of course, be enforced.

PERIPHERAL ARTERIAL DISEASE

Symptoms

In the peripheral vessels, as in the coronary, narrowing from atherosclerosis may take place gradually with incomplete obstruction or abruptly from thrombosis or embolism.

In the *upper extremities* clinically important lesions of vascular origin are rare even with grossly evident arteriosclerosis, although gangrene of the hands has been reported.

More frequent but still uncommon is occlusion of one of the arteries supplying the *abdominal viscera.* This condition is difficult to distinguish from other acute abdominal emergencies but should always be considered when suggestive symptoms occur in an older patient or in one with diabetes of long duration.

The clinical effects of peripheral vascular sclerosis are seen with overwhelming preponderance in the *lower extremities.* As with coronary disease among diabetics, the sex ratio is about one to one.

Symptoms are uncommon before age 50. They include intermittent claudication, coldness of the feet, pain, and paresthesias such as burning, tingling, and numbness. The latter may be due to vascular narrowing alone or to additional involvement of the vasa nervorum, as described by Woltman and Wilder, with symptoms resembling a true neuritis. On examination, the skin of the lower legs and feet is atrophic, shiny, and cool, with marked redness in the dependent position and a waxy pallor on elevation of the legs. Dependent rubor, it is to be noted, is a reliable sign of arterial insufficiency when venous stasis is not present; it is uncommon even in patients with varicose veins but otherwise normal vasculature. The pulsations of the posterior tibial and dorsalis pedis arteries are likely to be reduced or imperceptible; the same is true of the popliteal pulse, although this is often difficult to feel even in normal subjects. However, ischemic symptoms or frank gangrene may occur in patients with easily palpable pedal pulses, owing no doubt to the involvement of smaller vessels. On the whole, the color of the feet in dependency and the condition of their arteries as determined by palpation are as reliable in estimating the degree of impairment as the more refined methods of oscillometry, histamine wheals, and the like.

Treatment

VASODILATING DRUGS. The treatment of this condition is far from satisfactory. Vasodilating drugs by and large have given disappointing results, which is to be expected in the presence of rigid vessel walls. Their main justification lies in the possibility that they may favor the development of collateral circulation, but it is conceivable that the dilation of channels still able to respond only reduces blood flow in the narrowed vessels where it is most needed. The analgesic properties of alcohol recommend it above other dilating agents, but its hyperemic effect is exerted more on the skin than on deeper structures.

EXERCISE. The simplest and least expensive method of promoting circulation, and one that does as much good as any, is exercise. Walking should repeatedly be performed to the point of claudication. Buerger's exercises, with the feet alternately elevated over the head and depressed to the sitting position, should be carried out for 10 or 15 minutes twice daily. Patients unable to do this may use an oscillating bed. Mechanical methods for intermittent venous constriction have fallen into disuse. The feet should be kept warm at all times but not by the application of artificial heat, which is dangerous. Tobacco in any form should be forbidden.

ALCOHOL INJECTION. For intractable pain or threatened gangrene, local sympathectomy, alcohol injection of the lumbar paravertebral ganglia, or lumbar sympathectomy should be considered, but they are not often effective in older patients.

OPERATIVE PROCEDURES. Endarterectomy and by-passing operations ordinarily are not feasible in diabetic patients because of the generalized distribution of the intimal sclerosis and, in particular, the involvement of smaller vessels that do not lend themselves to these procedures. When there is reason to suspect occlusion of a larger vessel, and when pulsations of the more distal arteries are known to have been present previously, arteriography is worth performing in an effort to determine the location and extent of the obstruction and hence its operability.

Gangrene

According to Bell, gangrene is 156 times more frequent in diabetic than in nondiabetic individuals between the ages of 40 and 50, 85 times between 50 and 60 and 53 times between 60 and 70.

The lesion may be either "dry" or "wet" (infected). The *dry* or uninfected variety sometimes seems to start spontaneously as a hemorrhagic and later black blister on a single digit. Generally, however, trauma is the precipitating cause. This may be mechanical, thermal, or chemical. The common denominator is the swelling that follows injury and further impairs an already poor blood supply to the point where the tissues cannot survive. Corns and calluses, very frequently the sites of beginning gangrene, likewise reduce blood supply by compression of subjacent small vessels. In some cases a dark red spot composed of extravasated old blood can be seen deep in the center of the callus (Plate 38-1, facing page 634). Ulceration and infection often start here.

Gangrene of even a small area demands hospitalization and complete bed rest. Although healing, when it does occur, does not take place short of several weeks or months, and although hospital care for the

whole period may be economically impractical, two or three weeks of such care teach the patient how to carry on safely at home.

The less done to a gangrenous toe, when the process is dry, the better. The immediate aim of treatment is to keep it dry. The lesion should be left open to the air and should receive no wet dressings, antiseptics, or ointments. A foot cradle with supports well padded to prevent further injury to the foot serves to keep the bedding from touching the affected part. Heat, which only increases the metabolism of the tissues and hence their oxygen deficit, should not be used. Great care must be taken to avoid the development of pressure sores on the heels. This is best accomplished by placing a flat pillow under the calves, letting the feet just protrude over the end. Padded gauze "doughnuts," which in any case must not be secured with adhesive tape, never stay long in place and are worthless. The head of the bed is elevated on blocks, or the foot is depressed if a tilting bed is available, so that even with the leg pillow in place the feet are 6 or 8 inches below the heart. Thus gravity can be made to assist in maintaining blood flow. Patients who are well enough should perform Buerger's exercises two or three times daily. Arguments for and against vasodilating agents and sympathectomy are the same in the presence of gangrene as they are before it occurs. Pain is treated with salicylates and, if necessary, narcotics, but in some cases it will yield only to amputation. The prophylactic use of antibiotics is not justified. Diabetes, of course, should be strictly controlled.

If, after two or three weeks, the process has not spread and if the lesion remains dry and uninfected, the patient may be sent home to continue the same measures, including complete bed rest. The hope is that mummification and demarcation will progress to the point where the gangrenous area will separate by itself or with the aid of a minor operation. In many cases a major amputation can be avoided.

However, when dry gangrene of one or two toes goes on to invade a portion of the foot, the chances of success by conservative management are much diminished, and in proportion to the area involved. In such cases, high amputation is usually inevitable and there is no point in procrastinating.

In the *wet*, or infected, variety of gangrene the outlook depends upon whether the infectious or the vascular element predominates. With good pedal pulses, surgical drainage, and the use of appropriate antibiotics as dictated by culture and sensitivity tests, many feet can be saved. In such cases wet dressings, either before or after operation, are useful to prevent crusting and the imprisonment of infected material. A mixture of equal parts of saturated boric acid solution and 70 per cent alcohol

accomplishes this purpose and minimizes maceration of the skin, but even so, application should be intermittent rather than continuous. Heat in any form above 95° F. is strictly to be avoided. Roentgenograms should be taken of all infected feet to determine whether osteomyelitis is present. If infected bone does not respond to simple debridement, local amputation may be attempted. Following such operations healing is slow and prolonged care is necessary. As with any infection, the careful control of glycosuria is imperative and may require large doses of insulin.

If vascular impairment is severe and if the infection is deep and extensive, the prospect of cure by medical treatment is slight. In some cases it may be attempted for a week or so provided septicemia does not threaten. Once in a while a surprising recovery takes place.

PREVENTION. Much can be done to stave off gangrene in susceptible extremities. Measures to improve circulation, particularly Buerger's exercises and walking, should be taken regularly each day. The feet must be kept scrupulously clean by daily bathing, followed by massage and the application of lanolin if the skin is hard and dry, or alcohol if it becomes too soft and tender. Footwear must be changed daily. Stockings must not be allowed to wrinkle so as to produce blisters. Round garters should not be worn. Shoes should be neither too tight nor too loose and never too short; when new they should be worn for not more than an hour at a time until well broken in. Walking about the house unshod, especially in the dark, is strictly forbidden lest injury be incurred by striking the foot against the furniture. The greatest care should be taken to avoid frostbite and burns. Woolen socks should be worn in winter. No heat of any kind should be applied to the feet and the patient must be warned to test the bath water with his hand before he steps into the tub.

Patients with poor eyesight, tremor, or obesity of such proportions that they cannot see what they are doing should never trim their own toenails. The nails are allowed to grow out far enough to protect the ends of the toes and are cut straight across, not rounded. Ingrown toenails are not treated surgically but by stuffing a tiny pledget of cotton between the nail and the underlying skin.

The patient must never trim or pare calluses or corns, nor should any part of them be pulled or peeled off, since such procedures often cause bleeding. The feet should be soaked for 20 minutes in luke-warm, soapy water and the softened epithelium rubbed off with a coarse towel. The use of patent corn plasters or other keratolytic agents is likely to damage the surrounding normal skin. Persistent calluses under the metatarsal heads may be helped by the attachment of transverse leather

bars to the soles of the shoes an inch or so posterior to the point of maximum pressure. Ulcerated callosities are treated by abstinence from weight-bearing, and by the application of wet dressings and repeated cautious paring *by the physician* after they have been well softened by soaking. Healing is greatly prolonged unless the hyperkeratotic skin is almost completely removed. Sometimes these measures are best carried out in the hospital. As the ulcer fills in, exuberant granulation tissue is cauterized with a silver nitrate stick.

Cuts and abrasions should receive a mild antiseptic such as Zephiran or Merthiolate and should be covered with a thin sterile bandage. In no case should adhesive tape be applied to the skin. Epidermophytosis is treated actively with undecylenic acid (Desenex) as an ointment or powder.

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Chapter 39

RENAL DISEASE IN DIABETES

Robert M. Kark and Derek D. Gellman

It is surprising to recall that the pathognomonic glomerular lesions of diabetes mellitus, which are both common and serious, were described barely 20 years ago. Until the middle of the nineteenth century many physicians believed that diabetes was a primary disease of the kidneys; indeed the Dewey Decimal Classification, the cataloguing system used by most medical libraries, continued to list it as a renal disease until 1951. From the time of Richard Bright it had been recognized that "nephritis" was very common in patients with diabetes, and renal tubular lesions had been described by Armanni as early as 1875. For many years these were regarded as the most valuable diagnostic evidence of diabetes available post-mortem. Presumably the kidneys of many diabetics had been examined with great care by many pathologists looking for the tubular lesions, yet it was not until 1936 that Kimmelstiel and Wilson observed and described in 8 patients (7 of whom had diabetes) the unique nodular glomerulosclerotic lesion that now bears their names.

While it is true that any renal disease may occur in patients with diabetes, the lesions commonly found are:

1. Arteriosclerosis of the renal artery and its branches
2. Arteriolosclerosis

3. Diabetic "glomerulosclerosis"
   a. Nodular
   b. Diffuse*
   c. Exudative*
4. Tubular deposition of glycogen, fat, and mucopolysaccharides
5. Acute and chronic pyelonephritis

In addition, the following conditions, though not common, occur more frequently in diabetic patients than in the general population.

6. Necrotizing renal papillitis
7. Acute tubular necrosis
8. Toxemia of pregnancy

DIABETIC NEPHROPATHY

By "diabetic nephropathy" we mean the complex picture of arterial, arteriolar, glomerular, and tubular lesions that at present appears to be the inevitable consequence of long-continued diabetes. It is impossible to determine accurately the incidence of these lesions. To do so would involve making renal biopsies on a large sample of the whole diabetic population. Autopsy studies have shown that between 17 per cent and 36 per cent of diabetic patients *dying in hospitals* have nodular glomerulosclerosis. Diffuse glomerulosclerosis and arteriolar hyalinization are even more common.

Histology and Pathogenesis

The descriptions of the lesions which follow are based upon a critical review of previous publications, and on our findings in some 70 renal biopsies and in a large number of autopsy specimens from patients with diabetes.

NODULAR DIABETIC GLOMERULOSCLEROSIS OF KIMMELSTIEL AND WILSON. This lesion is generally accepted as being pathognomonic of diabetes. Typically the lesions consist of spherical nodules 20–100 μ in diameter, situated at the periphery of the glomerular capillary tufts. They are laminated, and one or more layers of nuclei can be seen embedded around the circumference. In the early stages dilated capillaries filled with blood run around them or along their capsular aspect (Plate 39-1). The presence of reticulin fibers in the nodules can be demonstrated with the short Foot stain (Fig. 39-1). In addition, they contain, among other substances, mucopolysaccharides, lipid, and hemoglobin (Muirhead).

* The diffuse and exudative lesions do not contain collagen or fibrous tissue, and strictly speaking they should not be called sclerotic. Common usage suggests employment of these names until the nature of the lesions is accurately understood.

The pathogenesis and mode of progression of the lesions are not yet completely understood. We believe that further studies with the electron microscope will be necessary to elucidate their development. It appears to us at present that following dilatation of capillary loops and sometimes following aneurysm formation, there is deposition of an abnormal substance, probably a complex mucopolysaccharide, which may



FIG. 39-1. Nodular glomerulosclerosis. The two nodules at the right of the glomerulus contain reticulin fibers arranged in layers. There are no reticulin fibers at the left, where the glomerulus is affected by diffuse glomerulosclerosis Short foot reticulin stain (× 500.)

come from the blood stream. It first penetrates the capillary endothelial cells, the nuclei of which lie next to each other on the inner aspect of the capillary loops. As the nodules increase in size they expand toward Bowman's capsule, pushing the capillaries ahead of them. By this stage, basement membrane and epithelial cells are also involved. Eventually the capillary lumen is occluded and the nodule becomes completely hyalinized.

DIFFUSE DIABETIC GLOMERULOSCLEROSIS. This lesion was first described by Fahr in 1912, but it was Bell who emphasized its importance as a pathological feature of diabetes. Many workers do not accept it as a specific lesion of diabetes, but in our opinion it can usually be distinguished from the superficially similar lesions seen in membranous glomerulonephritis and arteriolar nephrosclerosis. We have seen in renal biopsies all gradations of diffuse diabetic glomerulosclerosis, from the very earliest stage, which had never been described in autopsy specimens, to the more severe forms which had previously been described. It is this experience which enables us to make the distinction from the other similar lesions. Diffuse diabetic glomerulosclerosis consists of a thickening of the capillary walls which, in contrast to arteriolar nephrosclerosis, affects the peripheral loops at an early stage (Plate 39-2). Both inner and outer aspects of the wall are involved, and in cross section the capillaries appear as thickened rings. Except in the very earliest stages of the lesion all the loops of an individual glomerulus are involved (though often not to the same degree) and it is usual to find the lesions widely distributed throughout the kidney. The thickening of the walls causes narrowing of the capillary lumina

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PLATE 39-1. Nodular glomerulosclerosis. The nodule is laminated and the nuclei are congregated around its edge. It is surrounded by a widely patent capillary, which has a thin and delicate basement membrane on the side facing Bowman's capsule. There is a minimal degree of diffuse glomerulosclerosis affecting some of the other lobules. Hematoxylin and eosin.

PLATE 39-2. Diffuse glomerulosclerosis. There is diffuse but uneven thickening of the capillary walls involving their whole circumference. (Compare Plate 39-1 in which the capsular aspect of the capillary surrounding the nodule is spared.) Many of the capillary lumina are separated from one another by the intervening material. There is very slight hyalinization of the arteriole. Hematoxylin and eosin.

PLATE 39-3. Diffuse glomerulosclerosis. A more advanced lesion than that shown in Plate 39-2. The glomerulus is almost completely ischemic, but it is still large and fills Bowman's space There is no tendency toward nodule formation. Hematoxylin and eosin

PLATE 39-4. Exudative glomerulosclerosis. Note the amorphous, intensely eosinophilic "crescent" overlying a small nodule, and the capsular "drop." There is some diffuse glomerulosclerosis elsewhere in the glomerulus. Hematoxylin and eosin.

PLATE 39-5. Fat in tubules Notice that in addition to the relatively large fat droplets there are very fine fat "granules" more uniformly distributed throughout the tubules. Oil red "O"

PLATE 39-6. Peritubular hyaline "cuffing." Note the apparent thickening and smudging of the tubular basement membranes on the right, compared with the relatively normal ones on the left Periodic acid-Schiff.


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l eventually complete occlusion. The thickening appears to begin the basement membrane, perhaps as a result of deposition of abmal material. It then spreads to invade the endothelial cell cytosm and surrounds their nuclei, which become separated from the iina. Later the epithelial cell cytoplasm is also involved, so that the outlines disappear and only the nuclei can be distinguished (Plate 3). Special stains show that this lesion, like nodular glomerulosclerosis, contains lipid, mucopolysaccharides, and hemoglobin. However, reticulin fibers have never been demonstrated in diffuse glomerulosclerosis.

Diffuse diabetic glomerulosclerosis can be distinguished from the glomerular lesion of arteriolar nephrosclerosis by the following histological details: (1) The diabetic lesion commences in the periphery of the glomerulus, not at the hilus. (2) It does not have the "fibrillar" appearance, suggesting splitting of the basement membrane, which is seen in arteriolar nephrosclerosis. (3) When the glomerulus is completely involved with the diffuse diabetic lesion it remains large, instead of shrinking as it does with arteriolar nephrosclerosis. (4) The diabetic lesion does not contain collagen. The relationship between this lesion and nodular glomerulosclerosis is uncertain at present. Many workers believe that diffuse glomerulosclerosis is merely an early stage of nodular glomerulosclerosis, but it seems to us that their pathogenesis is probably different.

EXUDATIVE GLOMERULOSCLEROSIS. This lesion appears in the glomeruli only when the nodular and diffuse lesions are well developed. It is the least common and least specific of the three glomerular lesions. It can be seen as a clear, intensely eosinophilic substance, usually lying as a crescent or "drop" within Bowman's space but attached to the capsular surface of the glomerular tuft (Plate 39-4). It has the staining characteristics of fibrinoid and contains no collagen, but it does contain numerous other substances, such as triglycerides, cholesterol, mucopolysaccharides, etc. (Muirhead).

TUBULAR LESIONS. In 1875 Armanni described hyaline drops and vacuoles in the tubules of diabetics (Fig. 39-2). Later (1883) Frerichs wrote that Ehrlich, who was then an assistant in his laboratory, had identified the contents of the vacuoles as glycogen. The pathogenesis of the lesion has been studied by Oliver, who showed that the vacuoles occur principally in the *pars recta* of the proximal convoluted tubule at the corticomedullary junction. Presumably they are related to the reabsorption of abnormally large amounts of glucose lower in the nephron than usual. We have never found the Armanni-Epstein lesion

in a renal biopsy specimen, and it appears to be relatively uncommon in autopsy specimens at the present time, presumably because control of the primary disease has improved.

Small fat globules are not infrequently found in the glomeruli and tubules (Plate 39-5). Lipophages can sometimes be seen surrounding the tubules and in the glomeruli and blood vessels. Their presence implies longstanding hyperlipemia.

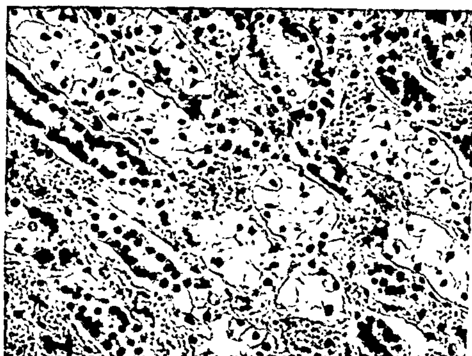


Fig. 39-2. Armanni-Epstein lesion. Many of the proximal tubular epithelial cells appear to be completely empty apart from their nuclei. The empty vacuoles represent the space previously occupied by glycogen, which was dissolved out during the process of fixation. Hematoxylin and eosin. (× 150.)

Peritubular hyaline cuffing was first observed in diabetes by Fahr. The material stains with the periodic acid-Schiff stain and is probably a mucopolysaccharide akin to that found in the renal glomeruli and elsewhere in the body (e.g., surrounding the mammary ducts) (Plate 39-6). It is always found in association with glomerular changes, and the picture of nodules in the glomeruli with peritubular cuffing is reminiscent of what is seen in amyloidosis, from which condition it can be distinguished by its failure to take up the crystal-violet and Congo-red stains.

ARTERIOSCLEROSIS AND ARTERIOLONEPHROSCLEROSIS. The blood vessels of patients with diabetes are peculiarly susceptible to atherosclerosis; the vessels of the kidney are no exception to this. Bell found severe intimal disease in the small renal arteries in 83 per cent of 821 diabetics over 50 years of age whom he examined post-mortem. Hyalinization of the arterioles is equally common, frequently involving the efferent as well as the afferent arteriole, a finding almost peculiar to diabetes. It is possible that narrowing of the efferent arteriole sets the scene for the development of capillary aneurysms and nodules in the glomeruli (Ashton). Despite the frequency and severity of arteriolosclerosis, the kidneys of diabetics are rarely very small; it is unusual to see contracted and scarred arteriolonephrosclerotic glomeruli, as they are generally distended by the specific deposition of abnormal mucopolysaccharides.

Etiology

Most of the pathologic lesions of diabetes, including the specific renal lesions, can be explained on the hypothesis of widespread deposition, particularly in and around the blood vessels, of protein-carbohydrate complexes that are present in abnormal amounts in the blood of patients with the disease. The problem is to decide whether the lesions are complications of diabetes which might be avoided by perfect control or whether they are concomitant changes, the tendency toward which is inherited separately. This important matter, which should influence our philosophy of diabetic management, has recently been well discussed by Duncan. Unfortunately there is as yet no clear-cut answer.

We know of no incontrovertible evidence that the specific renal lesions of diabetes can be prevented by strict control of the disease. Joslin and Dunlop found a lower incidence of all complications in patients who had been well controlled than in those who had been poorly controlled, but even in the well-controlled group the incidence of complications was not negligible if the patients were followed for long enough. Furthermore, it is impossible to be certain that the groups were really comparable. Not all diabetics are alike. Those patients who were apparently not well controlled may have had a more severe, *less controllable* form of diabetes than the well-controlled patients had.

If diabetic glomerulosclerosis is a complication of diabetes per se, it should be possible to produce it in animals rendered diabetic by pancreatectomy or by other means. Changes in the kidneys of diabetic animals have been reported by a number of workers, including Lukens, who produced diabetes in a dog with injections of anterior pituitary extract; Rich, who gave rabbits cortisone in doses equivalent to about 200 mg. per day in an adult human; and Bloodworth and Hamwi, who

used alloxan and desoxycorticosterone in rabbits. Although it is difficult to judge from illustrations, none of these workers seems to have produced lesions identical with nodular or diffuse diabetic glomerulosclerosis as seen in man. Some of the experimental lesions resemble the exudative type of glomerulosclerosis, but this occurs in human diabetics only when the kidneys are severely involved by the other lesions. Recently Dulin and we have studied the kidneys of rats with long-standing alloxan diabetes treated with insulin and sulfonylureas. Although many showed basement membrane changes, they were not identical with the diffuse lesions found in human diabetics and no nodular lesions were seen. It may be that these animals would have developed the characteristic diabetic lesions if they had been maintained in the diabetic state for longer periods. However, the lengths of time concerned represented a high proportion of the normal life span of these animals, and in man glomerulosclerosis can develop within 4 or 5 years after the apparent onset of diabetes.

The work of Rich and Bloodworth and Hamwi, and Becker's report that he had produced retinal microaneurysms in rabbits by the administration of steroids, suggested that overproduction of adrenal steroid hormones might be the cause of the specific vascular lesions in diabetes in man. However, there is no demonstrable difference in adrenal cortical activity in diabetics with and without vascular lesions (Rifkin). Furthermore, we have studied renal biopsies and autopsy specimens from more than 75 patients who have had steroid therapy for systemic lupus erythematosus, rheumatoid arthritis, or other diseases, but have never seen lesions in these kidneys resembling those we have described in diabetics. The kidneys of patients with Cushing's disease show an unusual lesion which does not mimic those found in diabetes, but resembles some of the lesions produced by Bloodworth and Hamwi.

Glomerulosclerosis appears to be extremely uncommon in patients with diabetes of known etiology. We know of no reports of glomerulosclerosis in diabetes resulting from pancreatectomy, hemochromatosis, acromegaly, or Cushing's syndrome, and we have never seen it ourselves. Duncan has reported one patient with diabetes, apparently resulting from mumps pancreatitis, who developed nodular glomerulosclerosis. In addition, he collected reports of 5 patients with "acquired" diabetes who had retinal microaneurysms. This is important in view of Ashton's observations of similarities in the pathogenesis of capillary aneurysms and nodules in the retina and in the renal glomerulus, and of the close clinical association of the lesions in these two sites.

The rarity of glomerulosclerosis in "acquired" diabetes, and the dif-

ficulty of producing it in experimental animals, are points against its being a complication and in favor of its being a concomitant of idiopathic diabetes. Against this view, and in favor of the complication theory, are the apparent rarity of glomerulosclerosis before 1923, the year of the introduction of insulin, and the difference in incidence in different parts of the world. Dr. H. T. Hyman, *et al.* (personal communication), have recently reviewed the kidney sections of all the diabetic patients on whom autopsies were performed at the Mallory Institute of Pathology of the Boston City Hospital during the years 1899–1924. Of 42 cases, only one had nodular glomerulosclerosis. It is possible that many of the patients did not survive long enough to develop the lesions, the incidence of which increases with duration of the disease. However, this is an unlikely explanation, since nodular glomerulosclerosis may occur in "adult onset" diabetics, whose survival has not been greatly affected by the introduction of insulin. The incidence of renal involvement differs considerably in different parts of the world. In a series of 200 diabetic patients attending a teaching hospital in East Africa, no cases were found of established renal disease, and only one of gangrene (Dr. A. G. Shaper, personal communication). Of 145 diabetic patients attending the Tohoku University Hospital in Japan, 26.8 per cent had persistent proteinuria and 8.6 per cent had the Kimmelstiel-Wilson Syndrome (Dr. N. Kuzuya, personal communication). These latter figures are comparable to what might be found in the United States. The typical Japanese diet (low fat, high carbohydrate) is similar to that of East Africans, and differs from that of Americans. The common feature distinguishing Americans and Japanese from East Africans appears to be the availability of insulin, which raises the possibility that the lesions are caused by the kidneys' reaction to injections of exogenous insulin. However, we have found nodules in one patient who, we were quite sure, had never received insulin. There are also a number of reports of patients with typical nodular lesions in whom the diagnosis of diabetes was not made during life; presumably they too had never received insulin.

Symptoms, Signs, and Diagnosis

There are no specific symptoms of renal involvement in diabetes. In known diabetics the urine should be examined frequently for evidence of renal damage. Persistent proteinuria, white cells or casts, in the absence of infection, suggest that the diffuse lesion is well established (Fig. 39-3, Table 39-1). The presence of oval fat bodies and fatty casts indicates the development of severe tubular changes. Unfortunately none of these urinary findings are pathognomonic of diabetic

TABLE 39-1. URINALYSIS AND GLOMERULAR LESIONS

Urinalysis	Number of cases	Mean severity ("plus" units) of Nodular glomerulosclerosis	Mean severity ("plus" units) of Diffuse glomerulosclerosis
Normal	22	0.2	0.8
Excess WBC only (>5/HPF)	6	0.2	1.0
Moderate numbers of casts No excess RBC or WBC	16	1.0	1.7
Moderate numbers of casts Excess WBC (>5/HPF)	6	1.2	2.5
Large numbers of casts, with or without excess WBC	13	1.3	2.7

glomerulosclerosis. The only certain method of diagnosis ante mortem is by renal biopsy. Suspicions should be aroused by the finding of proteinuria, hypertension, and azotemia, and the presence of the

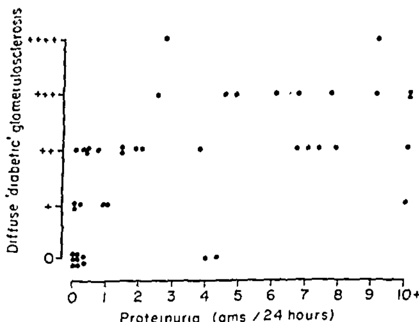


FIG. 39-3. The relationship between diffuse diabetic glomerulosclerosis and proteinuria. Each point represents the severity of diffuse diabetic glomerulosclerosis found in a single renal biopsy and the corresponding amount of protein in the urine. As the diffuse lesion becomes more severe, the quantity of proteinuria increases. The relationship is statistically significant ($p < 0.01$). (Figs 39-3 through 39-9 are reproduced by courtesy of Gellman *et al.*, *Medicine*, December, 1959.)

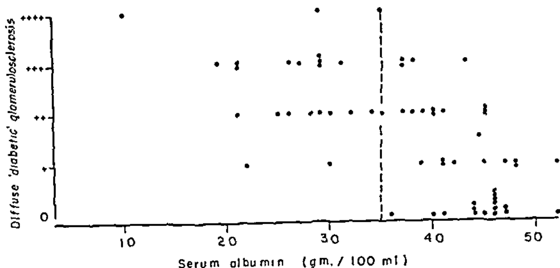


Fig. 39-4. The relationship between diffuse diabetic glomerulosclerosis and the level of serum albumin. As diffuse diabetic glomerulosclerosis becomes more severe, the serum albumin level falls ($p = 0.00001$).

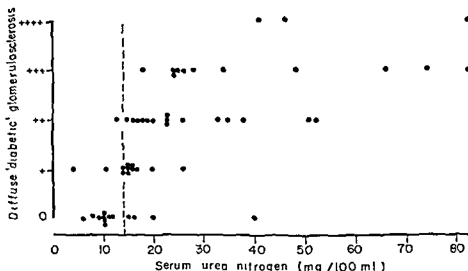


Fig. 39-5. The relationship between diffuse diabetic glomerulosclerosis and serum urea nitrogen. As diffuse diabetic glomerulosclerosis becomes more severe, the serum urea nitrogen level rises ($p = 0.00001$).

nephrotic syndrome makes the diagnosis virtually certain. (Of course, nephrotic syndrome owing to other causes can develop in diabetic patients, we have seen one such case.)

The degree of involvement of the kidney by diffuse glomerulosclerosis can be inferred from functional studies (Figs. 39-3–39-6). Analysis of the data indicates that a single study is not as satisfactory as a battery

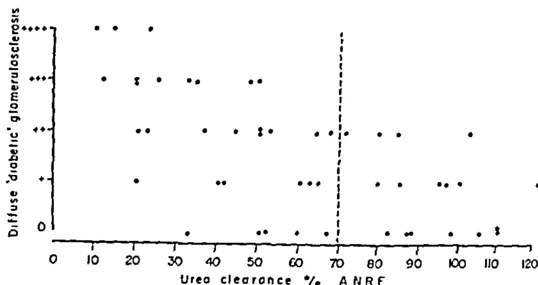


Fig. 39-6. The relationship between diffuse diabetic glomerulosclerosis and urea clearance. As diffuse diabetic glomerulosclerosis becomes more severe, the urea clearance falls ($p < 0.0001$).

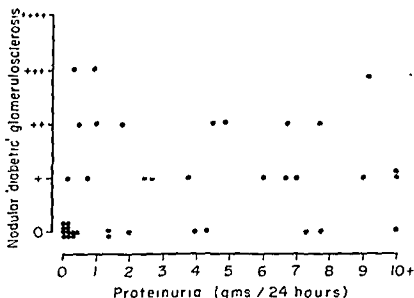


Fig. 39-7. The relationship between nodular diabetic glomerulosclerosis and proteinuria There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the quantity of protein in the urine ($p > 0.2$).

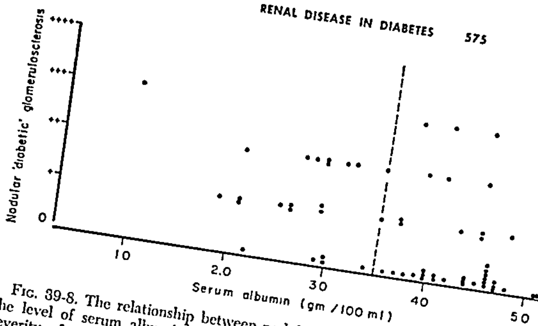


FIG. 39-8. The relationship between nodular diabetic glomerulosclerosis and the level of serum albumin. There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the serum albumin level ($p > 0.5$).

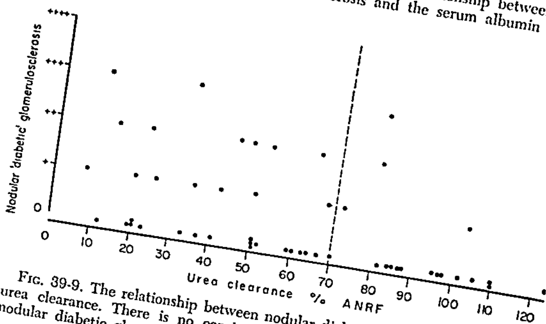


FIG. 39-9. The relationship between nodular diabetic glomerulosclerosis and urea clearance. There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the urea clearance ($p > 0.05$).

of renal function tests for predicting the severity of this lesion. No combination of tests can predict the severity of nodular glomerulosclerosis (Figs. 39-7–39-9). Even the nephrotic syndrome, erroneously called the "Kimmelstiel-Wilson Syndrome," can occur in diabetic patients who do not have nodular lesions. Conversely, less than 10 per cent of patients who do have nodular glomerulosclerosis also have the ne-

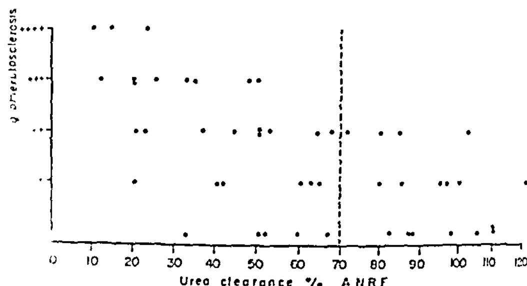


FIG. 39-6. The relationship between diffuse diabetic glomerulosclerosis and urea clearance. As diffuse diabetic glomerulosclerosis becomes more severe, the urea clearance falls ($p < 0.0001$).

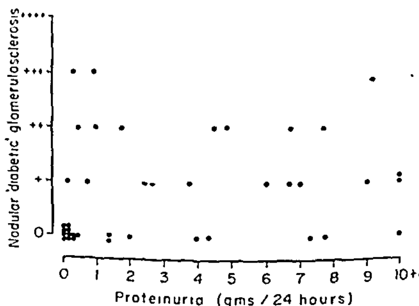


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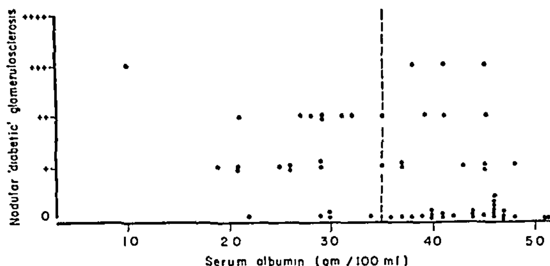


FIG. 39-8. The relationship between nodular diabetic glomerulosclerosis and the level of serum albumin. There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the serum albumin level ($p > 0.5$).

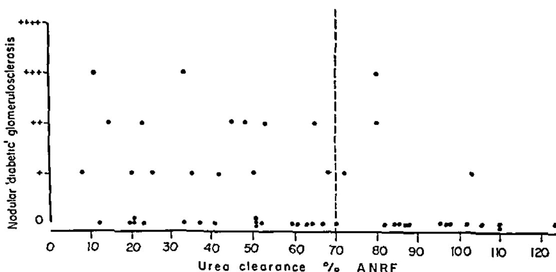
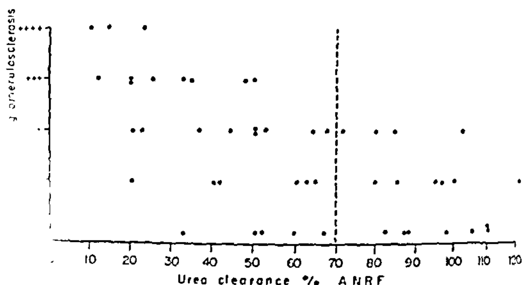


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39-6. The relationship between diffuse diabetic glomerulosclerosis and urea clearance. As diffuse diabetic glomerulosclerosis becomes more severe, the urea clearance falls ($p < 0.0001$).

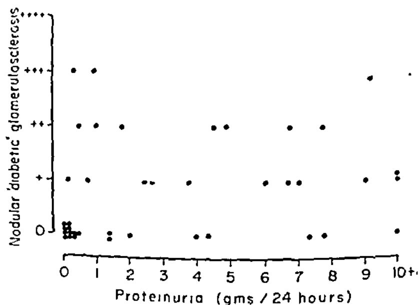


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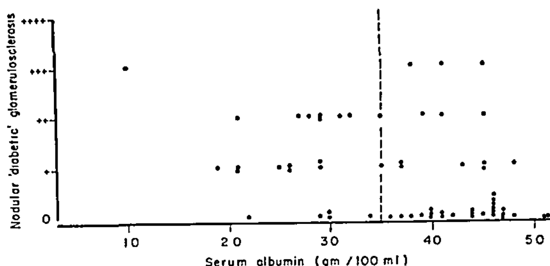


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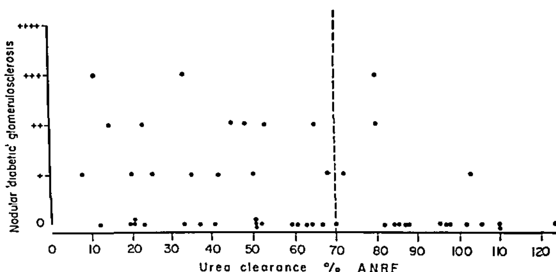


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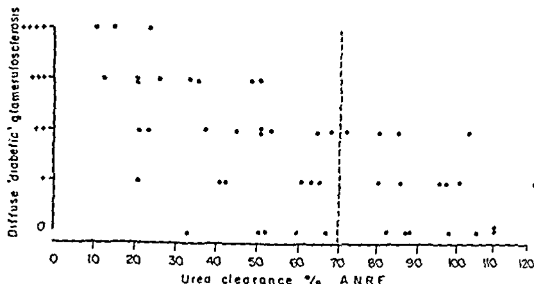


FIG. 39-6. The relationship between diffuse diabetic glomerulosclerosis and urea clearance. As diffuse diabetic glomerulosclerosis becomes more severe, the urea clearance falls ($p < 0.0001$).

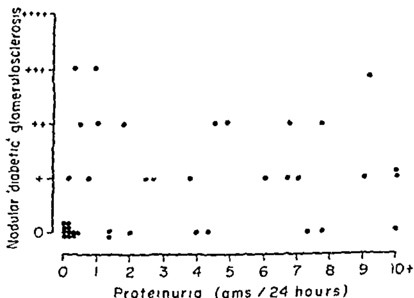


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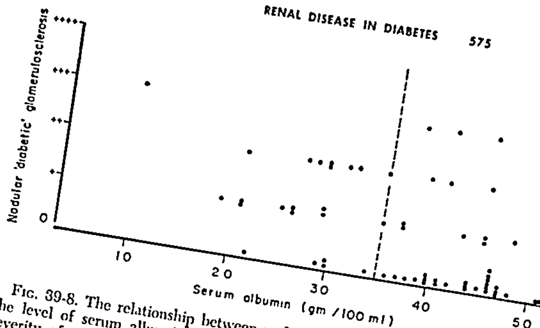


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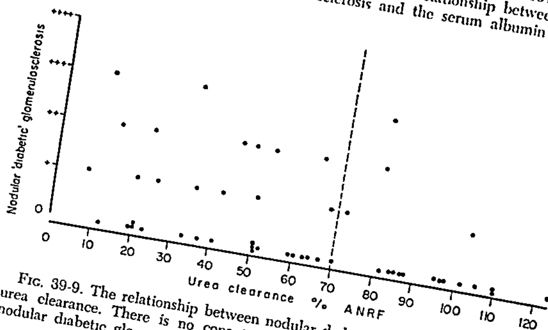


FIG. 39-9. The relationship between nodular diabetic glomerulosclerosis and urea clearance. There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the urea clearance ($p > 0.05$).

of renal function tests for predicting the severity of this lesion. No combination of tests can predict the severity of nodular glomerulosclerosis (Figs. 39-7–39-9). Even the nephrotic syndrome, erroneously called the "Kimmelstiel-Wilson Syndrome," can occur in diabetic patients who do not have nodular lesions. Conversely, less than 10 per cent of patients who do have nodular glomerulosclerosis also have the ne-

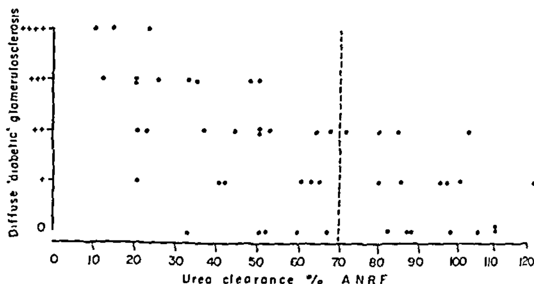


Fig. 39-6. The relationship between diffuse diabetic glomerulosclerosis and urea clearance. As diffuse diabetic glomerulosclerosis becomes more severe, the urea clearance falls ($p < 0.0001$).

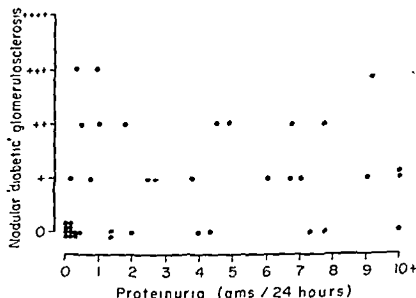


Fig 39-7 The relationship between nodular diabetic glomerulosclerosis and proteinuria. There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the quantity of protein in the urine ($p > 0.2$).

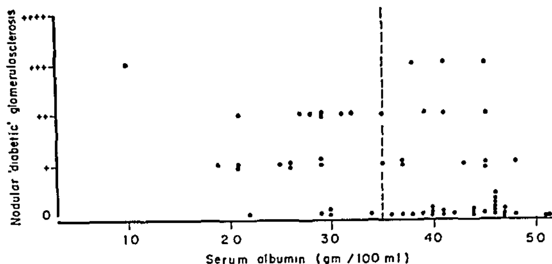


FIG. 39-8. The relationship between nodular diabetic glomerulosclerosis and the level of serum albumin. There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the serum albumin level ($p > 0.5$).

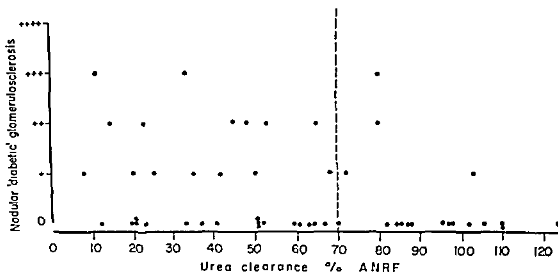


FIG. 39-9. The relationship between nodular diabetic glomerulosclerosis and urea clearance. There is no consistent relationship between the severity of nodular diabetic glomerulosclerosis and the urea clearance ($p > 0.05$).

of renal function tests for predicting the severity of this lesion. No combination of tests can predict the severity of nodular glomerulosclerosis (Figs. 39-7–39-9). Even the nephrotic syndrome, erroneously called the "Kimmelstiel-Wilson Syndrome," can occur in diabetic patients who do not have nodular lesions. Conversely, less than 10 per cent of patients who do have nodular glomerulosclerosis also have the ne-

phrotic syndrome. The common fault of diagnosing nodular glomerulosclerosis at the bedside might be corrected if the use of the term "Kimmelstiel-Wilson Syndrome" were abandoned. It is *diffuse* glomerulosclerosis, not nodular glomerulosclerosis, which is closely associated with renal failure and proteinuria and its consequences in diabetes (Gellman).

Occasionally, patients not previously known to have diabetes present in renal failure, without glycosuria, and are subsequently found to have the glomerular lesions specific for diabetes. Clinicians have known for a long time that the development of renal disease in diabetes is sometimes associated with the reduction or disappearance of glycosuria. The mechanism of this is not clear; possibly the reduction of glomerular filtration is such that the tubules can still reabsorb all the glucose that reaches them. Some workers have suspected a true amelioration of the diabetic state with renal involvement, and others, an amelioration with the development of the nephrotic syndrome. Recent experimental work by Kalant tends to confirm this last impression.

Prevention and Treatment

There are no data available to indicate that strict control of diabetes in an individual patient can prevent or reverse the renal involvement. It appears that strict control may reduce the incidence somewhat. Whether perfect control will prevent renal involvement altogether is not known.

It is fashionable at present to consider the fat content of the diet, and especially the content of animal fats, in the causation of vascular diseases. However, the difference between diabetics with vascular disease and those without appears to lie not in the blood lipids but in the glycoproteins, which are higher in those with vascular disease (Introzzi). Nevertheless, we feel that many of the diabetic diets in common use in the U.S.A have too high a fat content, and we have therefore redesigned the diets used in our own clinics. These diets now provide approximately 30 per cent of calories as fat, with adequate amounts of the triglycerides of the unsaturated fatty acids, as much protein (within reason) as the patient can afford, and the remainder of the required calories as carbohydrate. We try to maintain our patients at or just below their ideal weight. We should emphasize that we have as yet no *evidence* that such diets will prevent the development of renal complications, but we feel they are advisable on general grounds.

Once severe renal involvement has occurred, the management is, in general, not much different from that of other varieties of chronic renal failure. The control of the diabetic state itself usually becomes easier,

but occasionally it is complicated by the tendency of the patients to swing between hypoglycemic reactions (possibly because the azotemia interferes with their appetites), and hyperglycemic and ketotic episodes (because urine sugar tests are no longer a reliable guide to the blood sugar level). A high proportion of patients will develop blindness in association with this type of renal failure; this is a severe degree of invalidism which taxes the physician's cheerfulness and resources. His main function must be to ensure that the patient drinks enough water to provide a maximal excretion of urine and dissolved waste products. If necessary, the patient must be encouraged to wake himself at night to drink. Unnecessary interference with the body's attempts at homeostasis is to be deplored, except under unusual circumstances. Acid-base balance should be controlled only sufficiently to prevent symptoms. Perfect balance cannot be achieved and should not be attempted. Vomiting can be controlled with chlorpromazine injections. The progress of azotemia can be slowed by reducing the protein content of the diet (but not below the requirements of daily wear and tear, 30 to 40 gm./day) to keep pace with the kidneys' diminished ability to excrete nitrogenous waste products. Regular administration of aluminum hydroxide gel will reduce the absorption of phosphates. The total caloric intake must be maintained.

If protein loss in the urine is excessive (relative to the protein intake) and prolonged, the nephrotic syndrome will develop. By the time this occurs azotemia is usually severe and it is difficult to give the high protein diets usually indicated in the treatment of the nephrotic syndrome. The edema is often very resistant to all types of therapy, though prolonged sodium restriction and diuretic therapy may promote a diuresis. Chlorothiazide is probably the diuretic of choice at the present time.

There is no known method of reversing the pathologic process of diabetic glomerulosclerosis. The effectiveness of the rice diet and other low salt diets is not proved. A few reports of alleviation of diabetic vascular disease following pituitary ablation or adrenalectomy have been published but on the whole the results of these drastic operations have not been very encouraging and they need much more study before they can be considered as anything but experimental procedures.

INFECTIONS OF THE URINARY TRACT

Diabetes is frequently complicated by infection of the urinary tract. Of a random series of 55 male diabetic outpatients studied by us in a county hospital clinic, 11 (20 per cent) had infected urine. This inci-

dence approximates that found by others. None of these patients had symptoms suggestive of urinary tract infection.

Acute and Chronic Pyelonephritis

Infection in the kidneys often precipitates ketosis and coma in diabetics and it should be suspected in every patient with ketosis. Acute pyelonephritis is usually easy to recognize clinically because of high fever, pain and tenderness in the loin, and dysuria. The responsible organism can usually be obtained from the urine and sometimes from the blood. Chronic pyelonephritis is much more difficult to diagnose. Carefully collected clean-voided specimens should be examined microscopically and cultured quantitatively on tryptose-phosphate agar and E.M.B. plates (catheterization, which always introduces organisms into the urinary tract, should be avoided, if possible). A growth of 10^5 or more organisms per cubic centimeter is considered diagnostic of urinary tract infection. Lesser growths may be contaminants. Small numbers of white cells in the centrifuged urinary deposit (up to 5 per high-power field) are frequently found in patients with diabetic glomerulosclerosis in the absence of infection, but larger numbers of pus cells imply infection somewhere in the urinary tract. In the presence of infection, the finding of Sternheimer or "glitter" cells is strongly suggestive of pyelonephritis. Urography, excretion or retrograde, may provide additional clues, but often the diagnosis of chronic pyelonephritis can be made with certainty only by renal biopsy and culture of renal tissue.

Acute and chronic pyelonephritis are frequently stated to be "common" in diabetes, but just how common is difficult to determine. Robbins found evidence of acute pyelonephritis in 19.5 per cent of 307 autopsies on diabetics, and in one third of these it was thought to be the cause of death. This was more than four and one-half times its frequency as a cause of death in nondiabetics. We have been unable to find many statistics on the frequency of chronic pyelonephritis at autopsy, perhaps because of the difficulty of making the diagnosis in the presence of other chronic renal disease. It surprised us to find that none of our renal biopsies in diabetic patients contained definite evidence of chronic pyelonephritis if the criteria of Weiss and Parker were strictly applied. In about 10 per cent there was suggestive histological evidence. Nine of the patients who had had biopsies died at intervals of from 2 weeks to 18 months after the biopsy, and in these there was suggestive though not conclusive evidence of pyelonephritis in six (67 per cent). Two possible explanations for the discrepancy in these figures come to mind. First, sampling of the kidney with the biopsy needle may not be adequate to diagnose pyelonephritis. Second, pyelonephritis may develop as a terminal event in the diabetic. Yet a third

possibility is that the use of antibiotics has reduced the incidence of pyelonephritis.

It has been our experience that infection of the kidney in diabetic patients is difficult to eradicate with the usual 14-day course of antibiotics. Treatment should be vigorous and prolonged, and its effectiveness should be checked by repeated urinalyses and cultures. The choice of antibiotic or chemotherapeutic drug is determined by the results of sensitivity tests on the infecting organism. We are at present using a regimen of long-term specific therapy, up to three months, and following this with acid antiseptics such as mandelic acid and methionine.

Necrotizing Renal Papillitis

A rare but serious complication of pyelonephritis in diabetes is *necrotizing renal papillitis* (Lagergren). The condition is not confined to diabetes, but occurs in association with all types of acute infection, especially if obstructive lesions of the urinary tract are present. The pathogenesis of the condition is obscure, but it is generally thought to be due to ischemic necrosis of the renal papilla consequent upon overwhelming infection. The condition is usually diagnosed only at autopsy, but it should be suspected in diabetic patients who have fever, hematuria, renal colic, and advancing azotemia. The pyelographic appearances are characteristic, and sloughed portions of renal papillae may be found if the urine is strained through fine muslin. They should be searched for repeatedly. Although subacute and chronic forms of necrotizing renal papillitis may be commoner than was once thought, the patients are often desperately ill, and vigorous antibiotic therapy may have to be initiated before the results of cultures and sensitivity tests are available. Knowledge of any organisms that have infected the patient's urinary tract in the past will help determine the choice of drug. Antibiotics that have been administered in the previous few months (to which the organism may have acquired resistance) should be avoided if possible. If treatment has to be entirely empirical, intravenous chloramphenicol and erythromycin should be used. If the patient is not doing well, the regimen should be changed when sensitivity tests are completed. *If the organism is known to be sensitive,* streptomycin (combined with alkalis and sulfonamides) *is the drug of* choice, but it should not be used empirically. Streptomycin is effective in an alkaline urine only.

ACUTE TUBULAR NECROSIS

Acute tubular necrosis (so-called lower nephron nephrosis) may occur as a complication of diabetic coma. Usually it develops following

prolonged episodes of hypotension and shock, but occasionally no sound basis can be found to explain the renal shutdown. The possibility that the condition may be due to acute potassium depletion should be considered, since it is now well known that depletion of this electrolyte produces functional and histological changes in the kidney ("potassium depletion nephropathy").

TOXEMIA OF PREGNANCY

Diabetic mothers are particularly prone to develop toxemia of pregnancy—pre-eclampsia. Specific, reversible glomerular lesions are found in this condition. The glomeruli are large and ischemic, and the epithelial cells, basement membrane, and endothelial cells are edematous and swollen. The clinical manifestations are hypertension, proteinuria, and edema. Intermittent retinal vascular spasm may be seen. If untreated, convulsions may occur (eclampsia). The pregnancy should be terminated. This usually results in remission of the clinical findings, and the renal lesions revert to normal unless there is thickening (as opposed to mere edema) of the basement membrane, or unless, as is commonly the case in diabetics, there are other permanent changes.

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Chapter 40

OCULAR COMPLICATIONS OF DIABETES MELLITUS

Bernard Becker

The period of optimism that followed the discovery of insulin was reflected in the thinking and initial findings of ophthalmologists. Thus, the decrease in incidence of diabetic cataracts paralleled the dramatic decline in death rate from diabetic coma. It soon became apparent, however, that diabetes was a considerably more complex disease process than mere insulin deficiency. The problems of the late vascular complications of diabetes remain far from resolved. The distressing handicap of diabetic retinopathy constitutes a major threat to young diabetics as they reach their most productive years.

DIABETIC RETINOPATHY

Diabetic retinopathy is a distinct pathologic entity that can be recognized clinically and distinguished readily from other retinal vascular diseases. Unfortunately, this disease process has been repeatedly confused with other retinal pathology. In recent years, the study of flat preparations of whole retinas and the visualization of their entire vascular trees has greatly advanced knowledge about the nature of retinal vascular diseases. It would seem worthwhile, therefore, to outline the

basic pathology of diabetic retinopathy, and to correlate autopsy findings with the clinical progression of the disease as viewed ophthalmoscopically.

Pathologic and Clinical Picture of Diabetic Retinopathy

The first and most characteristic pathologic change in the retina in diabetic retinopathy is the appearance of discrete saccular aneurysmal dilatations of capillaries (Fig. 40-1). These are occasionally thin-walled,

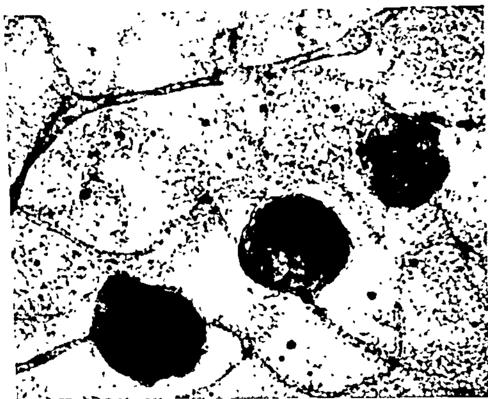


FIG. 40-1. Flat preparation of human diabetic retina stained with periodic acid-fuchsin. Pathologic appearance of patent and hyalinized capillary aneurysms.

but frequently lamination of hyaline material just outside the endothelium results in a thickened wall. Such material contains one or more mucopolysaccharides, which stain brilliantly with periodic acid-fuchsin. The aneurysms tend to occur in crops and appear ophthalmoscopically as clusters of tiny discrete red spots with a predilection for the deeper (inner nuclear) layers of the retina, especially in the perimacular region (Fig. 40-2). They persist for months and then may disappear or become hyalinized, lamellated polysaccharide nodules. Such hyalinized aneurysms may appear as small round white spots in the retina and are

to be distinguished from drusen as well as from the exudates of the next stage of this disease. In some instances, visible dilation or fusiform enlargements of the veins are seen in this early stage of retinopathy, but more often such changes are not noticed until much later in the disease process.

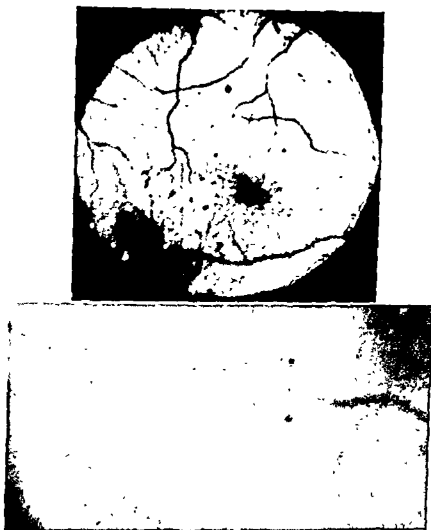


FIG. 40-2 Fundus photographs demonstrating ophthalmoscopic picture of capillary aneurysms in diabetic retinopathy.

The second stage of diabetic retinopathy consists of coalescing areas of aneurysms with surrounding intraretinal hemorrhages and exudates (Figs. 40-3 and 40-4). Some of the hemorrhages and exudates may be attributed to leakage of red cells and proteins through the walls of the capillary aneurysms. The hemorrhages are often deep in the retina and

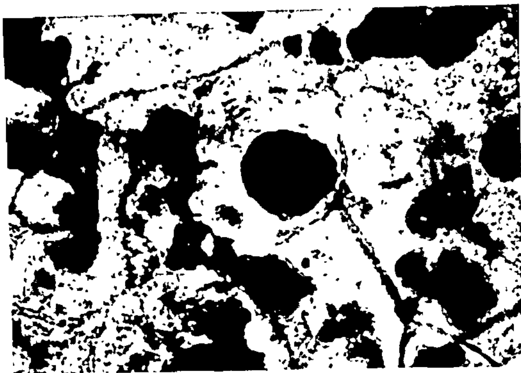


Fig. 40-3. Flat preparation of human diabetic retina: capillary aneurysms with surrounding hemorrhages and exudates.



Fig. 40-4. Fundus photograph of ophthalmoscopic picture of diabetic retinopathy with capillary aneurysms and surrounding hemorrhages and exudates.

therefore round in shape. At this stage of the disease, distortion of capillary patterns and venous distention are commonly seen. In most instances the disease progresses irregularly for long periods of time in this second stage with periods of exacerbation and spontaneous remission. It is important to realize that this is the natural course of the disease, and not to attribute the occasional improvement seen to the particular mode of therapy employed. It is evident that the chance hemorrhages that interfere with foveal function will produce enormous



Fig. 40-5. Fundus photograph of ophthalmoscopic picture of retinitis proliferans.

changes in vision, and that visual acuity is not in itself an adequate index of progression or regression of the disease.

At some variable period of time in approximately 25 per cent of cases, the third stage of the disease may be ushered in dramatically by the sudden loss of vision associated with preretinal hemorrhages breaking into the vitreous. Such hemorrhages resorb slowly and are associated with and frequently followed by fibrovascular organization leading to the condition known as retinitis proliferans (Fig. 40-5). The new-formed capillary tufts extending into the vitreous are themselves often the source of new hemorrhages, leading to further proliferation. This

stage of the disease has a particularly poor prognosis. Eyes so damaged usually lose all vision either from contraction of the vitreous fibrous bands leading to retinal detachment or from the more dreaded and extremely painful hemorrhagic glaucoma that ensues. In some instances neovascularization of the retina and even filmlike proliferative changes into the vitreous may occur following less well-defined hemorrhagic episodes, or occasionally even without evidence of pre-existing bleeding into the vitreous.

It is important to emphasize that all stages of the retinal disease can occur in the absence of associated hypertension or atherosclerosis. Although arteriolosclerotic and atherosclerotic lesions frequently complicate the picture, they are not a part of the basic pathology of diabetic retinopathy, nor is there evidence that they play a causal role in its pathogenesis.

Differential Diagnosis

Capillary aneurysms in the retina are not entirely specific for diabetic retinopathy, just as the Kimmelstiel-Wilson lesion of the kidney is described occasionally in nondiabetics. Capillary aneurysms are seen in a variety of diseases involving retinal tissue damage from inflammation, venous occlusion, etc. They are also found in patients with pernicious anemia, sickle-cell disease, and adrenal cortical hyperplasia, with long-term intensive corticosteroid therapy; and occasionally in the retinal periphery of apparently normal eyes. The aneurysms that occur following periphlebitis, sickle-cell disease, and other venous occlusions may be followed by hemorrhages into the vitreous and retinitis proliferans.

Aneurysms secondary to vein occlusion can usually be distinguished readily from those of diabetes. Vein occlusion is usually unilateral and the capillary aneurysms are fusiform, beaded, and varicose. The lesions are localized to the involved segment in the case of branch occlusion. The massive hemorrhagic picture of central vein occlusion is most characteristic. The diabetic microaneurysms are saccular, occur in apparently uninjured portions of the retina, and are bilateral. However, vein occlusions often do occur in eyes with pre-existing diabetic retinopathy, and this has been a cause of some confusion.

The changes in sickle-cell anemia, and more especially in those patients with the hemoglobin S-C trait, consist of venous dilatations, stasis, occlusions, tortuosity, capillary aneurysms, and hemorrhages. Hemorrhages into the vitreous, neovascularization, and proliferative changes may be observed. Both hemoglobin S-C disease and retinal periphlebitis (Eales's disease) tend to involve the retinal periphery first,

whereas, in contrast, the characteristic diabetic lesions are first observed in the posterior pole. The diagnosis of sickle-cell disease is suggested in Negro patients especially with a family history of this trait, and can be established by sickle preparations and by paper electrophoresis of hemoglobin.

Following prolonged administration of steroids, some nondiabetics develop retinal changes that cannot be distinguished from diabetic retinopathy. These may vary from the appearance of a few saccular

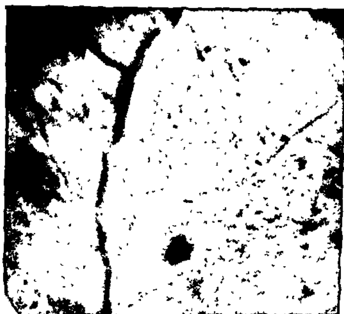


FIG. 40-6. Fundus photograph of ophthalmoscopic picture of retinopathy in a patient treated with steroids. Note enormous number of capillary aneurysms with relatively little hemorrhage or exudate.

aneurysms in the macular region to the involvement of the entire retina by innumerable scattered capillary aneurysms, hemorrhages, and exudates. All this may occur in the absence of diabetes (including negative glucose tolerance tests while on steroids) or evidences of renal disease.

An example of the marked form of this steroid-induced disease process was a 56-year-old man* with pemphigus who received corticotropin, cortisone, and prednisolone in large doses for a period of 4 years. Clinically, he had a picture identical with marked diabetic retinopathy. Figure 40-6 is a fundus photograph of the patient's right eye. At the time

* Patient seen through the courtesy of Dr. Marvin Rosecan.

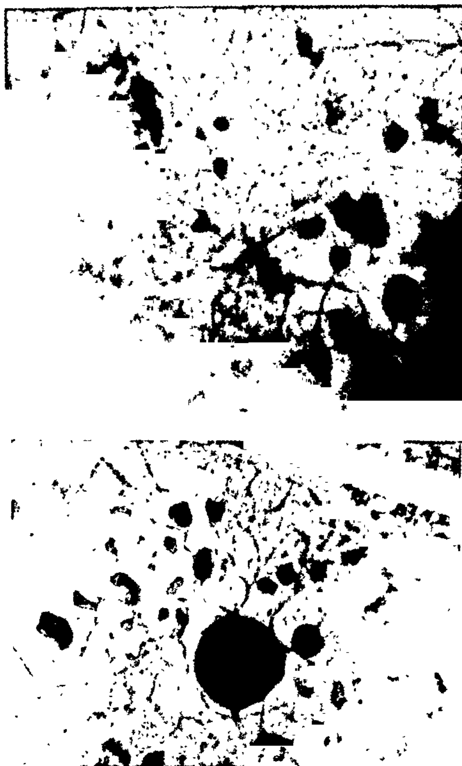


FIG. 40-7. Flat preparation of retina of patient in Fig 40-6: hyalinized and patent capillary aneurysms

of his death from a myocardial infarction, flat preparations of the retina confirmed the clinical findings of patent and hyalinized capillary aneurysms (Fig. 40-7). At no time during his course did he present glycosuria, hypertension, albuminuria, or evidence of impaired renal function.

Although pictures simulating diabetic retinopathy are seen in a number of other disease processes, the distribution of the capillary aneurysms and appearance of the vessels permit a tentative diagnosis. The laboratory data and other clinical findings usually establish the cause. It is important to emphasize that a normal blood sugar and the absence of glucose in a urine sample are not sufficient evidence to dismiss the diagnosis of diabetes mellitus in a patient with retinal capillary aneurysms. Glucose tolerance tests are indicated whenever these changes are observed, and occasionally have revealed mild abnormalities only after repeated testing.

Relation of Diabetic Retinopathy to the Nephropathy and Neuropathy

There are remarkable similarities between the clinical histories, the histologic appearances, the lipid deposits, and the staining characteristics of the lesions of diabetic retinopathy and the renal glomerular nodules described by Kimmelstiel and Wilson. Furthermore, it has been demonstrated repeatedly that diabetic retinopathy is found at autopsy in almost every diabetic patient with Kimmelstiel-Wilson renal lesions. On the other hand, patients without diabetic retinopathy rarely have renal lesions of the Kimmelstiel-Wilson type. In those patients with typical advanced diabetic retinopathy at autopsy who fail to demonstrate renal lesions on routine sections, serial sections of such kidneys usually reveal the typical Kimmelstiel-Wilson nodules. Recent electron microscopic data of Bergstrand and Bucht further serve to establish the histologic similarities of the retinal and glomerular lesions as joint manifestations of the same capillary vascular disease.

Diabetic neuropathy, like the retinopathy and the nephropathy, is a poorly understood complication of diabetes mellitus that is more closely related to the duration of the diabetes rather than to its severity. The neuropathy also appears to be on the basis of changes in small vessels with ischemic lesions in the nerve. A recent exploration of the relationship between diabetic retinopathy and diabetic neuropathy revealed a remarkable statistical correlation of the occurrence of these two disease entities as diagnosed clinically. Thus, over 90 per cent of patients seen with a clinical diagnosis of diabetic neuropathy had diabetic retinopathy. Recently Fagerberg has demonstrated PAS staining material in the walls of the small vasa nervorum of the sural nerve in diabetics with neuropathy. These capillary lesions closely resemble those seen in the ret-

inas. It, therefore, becomes apparent that we are dealing with a triad of complications of diabetes: neuropathy, nephropathy, and retinopathy.

Importance of Clinical Recognition of Diabetic Retinopathy

The importance of the recognition of the fundus picture of diabetic retinopathy by the physician is becoming more and more apparent. Diabetic retinopathy may be the first evidence suggesting the diagnosis of diabetes mellitus. Since the lesions of diabetic retinopathy are often associated with Kimmelstiel-Wilson changes in the kidney, the careful use of the ophthalmoscope alerts the physician to the early detection of this type of renal disease, perhaps before any clinical or laboratory signs are evident. On the other hand, the absence of retinopathy in a diabetic with albuminuria, edema, and hypertension should suggest other etiologic bases for the renal disease. Similarly, the close correlation of the retinopathy with the neuropathy of diabetes is of value in the differential diagnosis of this form of neuropathy. What is even more important, the observation of the lesions ophthalmoscopically in the living patient affords the physician the unparalleled opportunity of following the progression of such complications, and of evaluating the results of therapeutic endeavors.

Increasing Incidence of Diabetic Retinopathy

The amazing increase in incidence of the triad of complications of diabetes is out of proportion to the improved survival rate of diabetics. It presents one of the outstanding challenges to the medical profession today. Thus, Wagener noted diabetic retinopathy in 8.3 per cent of diabetics in 1921, 17.7 per cent in 1934, and 30.6 per cent in 1945. At present in large diabetic clinics the incidence of retinopathy runs as high as 50 per cent of all diabetic patients, if the patients' pupils are dilated and the earliest lesions are looked for carefully. Among those patients with diabetes of over 15 years' duration, over 75 per cent have some stage of retinopathy and some 25 to 30 per cent of these have retinitis proliferans. In fact it is becoming alarmingly apparent that almost all the steadily increasing number of young diabetic patients who are being kept alive by the use of insulin and supportive therapy for 25 years or more develop ocular and related complications, and over 50 per cent of these have advanced to retinitis proliferans. Thus, there is a pressing need to increase our meager knowledge about the pathogenesis of this syndrome.

Diabetic Retinopathy and Pregnancy

Diabetic retinopathy has been noted to appear for the first time in nonhypertensive diabetics during pregnancy and to clear completely

following delivery. In addition, many diabetics with retinopathy have been observed whose ocular disease showed startling progression during the last trimester of pregnancy and subsequent regression postpartum.

Comparison of Diabetics With and Without Retinopathy

Extensive efforts have been made to compare diabetic patients with and without retinopathy in order to discover possible metabolic, nutritional, or endocrine differences between these two groups. Unfortunately, there is no general agreement among observers as to some of the significant differences between these two groups of diabetics. This stems in part from the variation in methodology of the investigators, but also from marked discrepancies in case selection and classification. Investigators oriented ophthalmologically select for their retinopathy group patients with early active disease, consisting mainly of fresh capillary aneurysms and retinal hemorrhages. This is of particular importance in view of the known episodic nature of the active lesions and longer periods of slow repair. Such patients usually have no visual disturbance whatsoever, and often can be recognized only following an exhaustive search with a brilliantly illuminated, well-focused ophthalmoscope and the patients' pupils dilated. The ophthalmologic groups tend to rule out from their series those patients with "burned out" retinopathy with proliferative and degenerative changes even though such eyes have the greatest visual impairment. Some other investigators acquire their data from series that include the advanced "inactive" eyes in the retinopathy group, and further obscure the issue by including those eyes with only an occasional aneurysm in the nonretinopathy group. Under these circumstances, regardless of refinement of measurements, existing differences become obscured.

DURATION OF DIABETES. Diabetic retinopathy is usually related to the duration of the diabetes. However, occasional instances are noted of the appearance of the retinopathy and nephropathy before other clinical evidence of diabetes. The latter finding, and the occasional patient free of retinopathy even after more than 25 years of diabetes, provide evidence that duration of the metabolic disorder is not the sole explanation of the retinopathy.

CONTROL. A number of studies indicate that diabetics with retinopathy have histories of poor control more often than those without retinopathy. It is not clear, however, whether this is related to the amount of effort made to regulate the diabetes rigidly or to inherent initial differences in the diabetic state and its ease of control. Evidence against there being a particular type of diabetes more susceptible to vascular damage is provided by the clinical occurrence of diabetic retinopathy in such

acquired varieties of the disease as follow total pancreatectomy, hemochromatosis, acromegaly, or Cushing's syndrome.

INSULIN REQUIREMENTS. In most series no differences in initial severity of diabetes or insulin requirements have been demonstrated between diabetics with and without retinopathy. However, some authorities believe that the young individual with severe diabetes is more likely to develop marked retinopathy. On the other hand, in some instances there appears to be an amelioration of the diabetes with reduction of insulin requirement in diabetics with advanced retinopathy. This may be related to lessened activity or to renal insufficiency and reduced food intake. Insulin requirements may also be reduced by other ill-defined effects of the nephrotic syndrome as demonstrated by Kalant in alloxan-diabetic rats made nephrotic.

POLYSACCHARIDES AND LIPOPROTEINS. There is an increased level of serum glucosamine, polysaccharides, and α-2 globulins in the serum of diabetics with retinopathy as compared to those without this complication.

Diabetics with retinopathy also have a higher plasma lipid levels than those without retinopathy, especially the S_f 12–20 class of circulating lipoproteins.

BLOOD COAGULATION DEFECTS. Diabetics with retinopathy have been found to have increased capillary fragility. In addition, abnormalities of platelet glycogen and mucopolysaccharides have been described in some diabetics. In several reported series, however, the clotting defects were not confined to the retinopathy group.

ADRENAL HISTOLOGY. The adrenals of diabetics with retinopathy have been demonstrated to weigh more on the average, to display more lipoid vacuolization, and to have a higher incidence of cortical adenomas than do the adrenals of diabetics without retinopathy.

CORTICOTROPIN EOSINOPENIA. Four hours following the administration of corticotropin, almost all diabetics with retinopathy demonstrate at least a 50 per cent fall in circulating eosinophils, much as do normal individuals However, approximately half of the diabetics with no evidence of retinopathy fail to show this response. These unresponsive diabetics do obtain a fall in circulating eosinophils following cortisone administration, indicating that the defect is not at the level of the eosinophil. Such diabetics thus resemble patients with Addison's disease or bilateral adrenalectomy.

Alloxan diabetic rats are more susceptible to a deficiency of pantothenate than are nondiabetic animals. Furthermore, pantothenic acid plays an important role in the biosynthesis of steroids and has been found necessary for the maintenance of the zona fasciculata of the

adrenal cortex of the rat. When pantothenic acid is administered to those diabetics without retinopathy who fail to obtain an eosinopenic response to corticotropin, many become responsive to the corticotropin. However, pantothenate deficiency in these diabetics is not marked enough to affect their ability to acetylate sulfadiazine.

GLUCOCORTICOID EXCRETION. Diabetics with retinopathy demonstrate large fluctuations in daily 17-hydroxycorticosteroid urinary excretion. In one series of carefully classified diabetics, those with retinopathy excreted significantly larger amounts of 17-hydroxycorticosteroids in their 24-hour urines than those without retinopathy. However, other observers fail to confirm differences in measurable known adrenal steroids in the blood or urine. These discrepancies may relate to the selection of cases, the criteria used in the diagnosis of retinopathy, or to the intermittent nature of the abnormal steroid output in diabetic retinopathy and the episodic nature of the disease process.

VITAMIN B_{12}. Serum levels of vitamin B_{12} are significantly higher in diabetics with retinopathy than in those without this complication. Furthermore, following the intramuscular injection of 50 μg. of vitamin B_{12}, diabetics with retinopathy excrete significantly more of the test dose in their urine than do diabetics without retinopathy. This is of considerable interest because of the previously mentioned finding of capillary aneurysms in some patients with untreated pernicious anemia. However, some observers do not find significant differences in urinary or serum B_{12} levels between the two groups of diabetics.

Experimental Lesions

The experimental production of capillary aneurysms has been best accomplished by inducing vein occlusions in otherwise healthy cats. In these experimental animals the innumerable patent and hyalinized aneurysms produced are found to be confined to the area drained by the vein occluded.

Attempts at the production of capillary aneurysms in rabbits have not been consistently successful. Thus, although one obtains occasional capillary aneurysms following vein occlusion or by the administration of adrenocortical steroids to alloxan diabetic animals, such changes cannot be produced regularly. Retinal lesions are rarely present even in animals that have the most marked capillary aneurysms and hyalinelike lesions in their renal glomeruli (e.g., following the administration of prednisolone to alloxan diabetic rabbits or to animals on diets not supplemented by vitamin B_{12} and aureomycin). The intravenous administration of lipids, polysaccharides, polyethylene balls, gum arabic, starch, γ-globulin, etc., have also failed to induce retinal capillary aneurysms.

This may be related to the very limited capillary vasculature of the rabbit retina. Unfortunately, other experimental animals have proved remarkably resistant to the experimental production by adrenal steroids or corticotropin of either the renal or ocular lesions of diabetes. Choline deficiency in rats with consequent lipid plugs in capillaries, has also failed to induce retinal lesions. However, as indicated above, capillary aneurysms have been seen occasionally in the human retina associated with long-term corticosteroid administration.

Theories as to Pathogenesis

A number of theories have been proposed to account for the pathogenesis of the capillary lesions in the retina as well as to explain the differences between diabetics with and those without retinopathy. Much of the thinking about the intimate mechanism of anuerysm formation in retinal capillaries rests upon the known production of such changes by venous occlusion. It is well known that retinal venous pressure is high, since veins are patent in spite of the normally high intraocular pressure. However, retinal capillaries have this same intraocular pressure surrounding them for support. Hypotheses vary largely as to the mechanism of the increased stasis at the venous end of the capillary and the relative importance of such factors as vascular pressure, local defects in the capillary wall, and vasoformative stimuli.

CIRCULATING FACTORS. Disorders of mucoid or fat metabolism with circulation and deposition of these factors in the venous end of capillaries have been offered as explanations for the renal and retinal lesions. Serum polysaccharides and lipids are elevated in diabetic retinopathy but these changes are nonspecific. Fat emboli are described and lipids, lipohyalin, and erythrocyte fragments are found in the glomerular nodules and retinal capillary aneurysms. Pregnancy, which often aggravates the retinopathy, also leads to elevations of glycoproteins and lipoproteins especially in the last trimester. Similarly, Lerman finds that cortisone administration results in elevated serum lipids and α-2 globulins in experimental animals. Platelet, red cell, or coagulation defects have also been proposed as possible mechanisms causing stasis or occlusion in venules. If platelet mucopolysaccharides contribute to the maintenance of the capillary wall, an attractive hypothesis can be developed based upon progressive abnormalities of this circulating factor in the diabetic. However, the specific localization in selected sites suggests some additional local factor. Therefore, the causative role of circulating factors in the morphogenesis of the capillary lesions remains to be explained.

LOCAL ALTERATIONS IN THE CAPILLARY WALL. Changes in the basement membrane of the capillary of the retina have been postulated as

vein occlusion, they do not appear to affect the progression of the retinopathic process. However, as indicated above, heparin has been demonstrated to prevent the lipemia and renal lesion produced by cortisone *in the rabbit.*

CONTROL OF DIABETES. Most ophthalmologists resort to the recommendation of careful diabetic control as the best means of handling diabetic retinopathy in the present state of our knowledge. However, rigid control has not been demonstrated to alleviate or decrease the rate of progression of established diabetic retinopathy.

Therapy with such oral hypoglycemic agents as tolbutamide also does not prevent or alter the vascular complications of diabetes.

OCULAR SURGERY. Surgery may be indicated in a limited number of instances of advanced stages of the retinal complication of diabetes. *Retinal detachment surgery in proliferative retinopathy has been unsuccessful* in the past. The availability of better techniques of scleral shortening procedures offers occasional opportunities for retaining or restoring vision for limited periods of time. Vitreous transplants may be indicated following hemorrhages into the vitreous, but only in those rare cases with subsequent remission of activity. X-ray therapy appears to be of little value. The hemorrhagic glaucoma associated with diabetic retinopathy may occasionally be controlled by cyclodiathermy.

THE LENS IN DIABETICS

The lens is a transparent structure of ectodermal origin, which has no nerve or blood supply and is nourished entirely by the aqueous humor. It has a very high protein content (35 per cent) and contains high concentrations of ascorbate and glutathione. There is a single layer of epithelial cells beneath its anterior capsule. Fibers are produced continuously at the equator and their incorporation within the lens results in its enlargement throughout life.

The lens maintains within itself high potassium (120 mM./kg. of water) and low sodium (25mM./kg. of water) concentrations. Like the red blood cell, when cooled it loses potassium to and gains sodium from bathing fluids. It can restore the K/Na ratio when rewarmed, provided that it has an adequate supply of calcium, energy derived from glucose, and the availability of active enzymes of glycolytic and oxidative metabolic pathways.

The lens has a low oxygen consumption, and this is confined almost entirely to the single layer of epithelial cells that contain the cytochromes. Glucose utilization by the lens is largely glycolytic (80 per cent to 85 per cent). However, because of the greater efficiency of the citric acid cycle and direct oxidative shunt pathways, which account for

the remainder of the glucose utilized, oxidation is responsible for at least 50 per cent of the energy used.

Alterations in the lens may result from (1) direct or indirect systemic effects, (2) qualitative or quantitative variations in aqueous secretion by the ciliary body, and (3) changes in the metabolism of the lens itself. In diabetes all three types of alterations may play a role in the changes induced in the lens.

In the alloxan diabetic animal, lens changes develop at a rate that is related to the degree of blood sugar elevation. Their production can be delayed by decreasing the blood sugar level not only by insulin but also by such nonspecific means as starvation, high fat diet or the administration of phlorizin.

Insulin also increases the rate of transport of glucose by the ciliary epithelium and thus affects the nature of the secretory product. Even a complete failure of aqueous secretion may be seen in diabetic coma, resulting in the ocular hypotony associated with this condition.

In vitro culture studies of the lens demonstrate that the uptake of glucose can be altered by insulin deficiencies and excesses. Thus, alloxan diabetes in the donor animal will decrease, and insulin administration in vivo will increase the subsequent uptake of glucose in the lens culture. However, in these experiments insulin had no effect on glucose utilization when added in vitro. It is clear that the lens affords an excellent tool for the study of various aspects of the disordered metabolism of diabetes.

Changes in Refraction and Accommodation

Weakness of accommodation and sudden changes in refraction occur most often in young diabetics. They are probably related to the effect of osmotic changes on the lens, but some of these symptoms may be related to metabolic and functional alterations in the muscles of the ciliary body. A sudden appearance of myopia or any rapid marked changes in refractive error may be the presenting symptom of diabetes, and therefore should arouse suspicion. In the known diabetic similar findings may serve as a warning sign of poor regulation. They tend to disappear with control of the diabetes.

Cataracts

Two types of cataracts are seen in diabetics: metabolic and senile. The metabolic cataract occurs in young people with severe diabetes. It is snowflake in appearance and starts in the subcapsular regions of the lens. The opacification may increase rapidly but may be temporary and reversible in its early stages. It is seen much less frequently since insulin has become available.

Senile cataracts in diabetics do not differ from those in the aged. Some observers believe them to appear earlier and occur with somewhat greater frequency than in the nondiabetic. The therapy of cataracts in the diabetic is much the same as in the nondiabetic. Cataract extraction should be recommended when vision becomes insufficient for the patient's needs.

IRIS COMPLICATIONS IN DIABETES

Iridopathy

The deposition of glycogen in the pigment epithelium of the posterior surface of the iris and the depigmentation of this layer is most characteristic of the diabetic. The friability of the pigment epithelium may result in pigment liberation into the anterior chamber, particularly when the eye is subjected to such surgery as cataract extraction. Loss of pigment often gives a vacuolated and moth-eaten appearance to the iris. It has no known pathologic significance, gives rise to no symptoms, and is not related to the lens or retinal complications of diabetes.

Rubeosis Iridis

Neovascularization of the anterior surface of the iris and the angle of the anterior chamber results in hemorrhagic glaucoma. In the diabetic the condition is almost always associated with the proliferative retinopathy. It may also follow central vein occlusion. The resulting glaucoma is most difficult to treat, but occasionally responds to diathermy cauterization of the ciliary body. The prognosis for retention of any vision in eyes with this condition is extremely poor.

LIPEMIA RETINALIS

This unusual retinal condition occurs very rarely in diabetics since the advent of insulin. It is also seen in nondiabetics and is dependent for its appearance on a high neutral fat content of the blood A light salmon color of the vessels appears at fat levels of approximately 3.5 gm. per cent, and a pink-white appearance of the retinal vessels is seen when the neutral fat level is above about 8 gm. per cent.

MISCELLANEOUS EYE CONDITIONS

Other eye conditions such as chronic simple glaucoma, iritis, optic and retrobulbar neuritis, primary optic atrophy, etc., are seen in the diabetic, but do not differ in incidence or appearance from those seen in nondiabetics.

SUMMARY

Ocular complications are associated with the acute direct metabolic aspects of the diabetic state (e.g., cataracts) as well as the long-term vascular changes (e.g., diabetic retinopathy). The eye offers opportunities for studying the alterations clinically under magnification, permitting early recognition, observation of progression, and evaluation of therapy. In addition the nature of the metabolic disorders may be explored experimentally in structures already layered, isolated, and dissected.

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Chapter 41

THE NEUROLOGIC COMPLICATIONS OF DIABETES MELLITUS

Fred Plum

INTRODUCTION

The neurologic problems of diabetics involve mainly the peripheral nerves. However, many diabetics suffer from at least transient functional muscular weakness and a few develop primary spinal cord lesions. Because of their high incidence of arteriosclerosis, diabetics are more prone than the rest of the population to cerebral vascular disease. Finally, the potential hazards of insulin therapy create for the diabetic an additional risk of damage to the nervous system.

MUSCULAR DISTURBANCES

Muscular weakness is prominent among the presenting complaints of patients with untreated diabetes. Those with incipient ketosis often complain of a particularly profound weakness and lassitude. These troublesome symptoms are unexplained. Hyperglycemia, per se, causes no acute impairment in neuromuscular or muscle function and the little morphologic evidence available indicates that the muscles themselves

are free from pathologic changes. Since hypopotassemia is rarely found in untreated diabetics, this cannot explain the weakness.

Muscle weakness, which may progress to severe generalized paralysis, sometimes develops during recovery from diabetic coma. Here, the pathogenesis is impaired neuromuscular transmission owing to hypopotassemia. Large quantities of potassium are lost during the osmotic diuresis that precedes and accompanies diabetic coma. With depleted potassium reserves, hypopotassemia may develop promptly if electrolyte-free fluids are relied upon during the time that insulin-induced glycogenesis shifts large quantities of potassium from the extracellular to the intracellular fluids. The prevention and treatment of this problem are discussed in detail in Chapter 37.

PERIPHERAL NEUROPATHY

GENERAL CONSIDERATIONS

Estimates of the frequency of peripheral neuropathy among diabetics vary widely according to the criteria employed for diagnosis. As many as 30 to 50 per cent of diabetics may show minor reflex changes and evanescent pains in the extremities. More troublesome and specific indications of peripheral nerve disease are less frequent, the incidence in most large series being less than 5 per cent of the total diabetic population (Table 41-1).

TABLE 41-1. THE FREQUENCY, AGE, AND SEX DISTRIBUTION OF DIABETIC NEUROPATHY

Author	*Total number of cases*	*Cases of neuropathy*	*Percentages*			
			Under 30	*30–50*	*50–70+*	*Males*
Broch and Klovstad	426	88 (20 7%)	14 8	13.6	70.4	46.6
Jordan	1,000	25 (2.5%)				
		120	5.1	(av. age	54.7)	42.5
Martin		150	5 3	25.3	69 4	47
Rudy and Epstein		100	5	18	77	34
Rundles	3,000	125 (4 2%)	15.2	32	52.8	55

Peripheral nerve disease is particularly prone to occur in the older diabetic and in the patient who has had relatively mild disease of several years' duration. However, a significant number of patients under 30 years of age develop neuropathy—often concomitantly with the onset of

other manifestations of generalized diabetes. Occasionally, the neuropathy becomes prominent while the diabetes is still latent or asymptomatic. Many workers have observed that peripheral neuropathy is prone to appear in previously untreated diabetics within one or two weeks after first starting insulin. In most large series, more women than men are found with peripheral neuropathy, but this sex difference is slight and probably not significant.

PATHOLOGY

Lesions in the peripheral nerves in diabetics have been found at all levels from the spinal intramedullary roots to the terminal filaments in the foot. Both diffuse and patchy degeneration has been noted with the lesion often affecting less than the full diameter of the nerve. Myelin destruction usually exceeds the disruption of axis cylinders. Inflammation is lacking but connective tissue proliferation may be prominent. Woltman and Wilder found thickening of the walls of the intraneural vessels in all their cases. Since these workers obtained specimens either from amputated extremities or from older subjects at autopsy, the general applicability of their findings to all cases of diabetic neuropathy has been doubted.

When one considers how long the disease has been studied, there is a remarkable paucity of detailed and comprehensive neuropathologic material on diabetic neuropathy. However, Fagerberg's preliminary report on biopsy material from the sural nerve in diabetics of all ages is of great potential interest. He found, in both younger and older diabetics with neuropathy, thickening in the vessel walls that gave a positive stain for mucopolysaccharides. Fagerberg tentatively concluded that these changes were specific for diabetes and similar to the vascular abnormality encountered in retinal and renal vessels.

ETIOLOGY AND PATHOGENESIS

Three principal hypotheses have been advanced to explain diabetic neuropathy: (1) That it is a specific nutritional deficiency. (2) That it directly results from abnormal carbohydrate or fat metabolism. (3) That it is secondary to vascular disease. Much has been written on the subject but critical data to support or refute the latter two of these hypotheses are still fragmentary.

Nutritional Deficiency

The notion of diabetic neuropathy being due to a specific lack of either vitamins or other external nutrients has now been almost universally surrendered. In the preinsulin years of dietary control, moder-

ate malnutrition was frequent among diabetics. The incidence of neuropathy has not lessened now that insulin permits most diabetics to eat an excellent diet. Almost every available vitamin and other specific food stuff has been tried both orally and parenterally in diabetics with neuropathy. Satisfactory evidence is lacking that any of these treatments has favorably affected the course of the disease.

Abnormal Metabolism

Many workers who have studied carefully the problem of diabetic neuropathy have concluded on clinical grounds that the disease must have its origin in either abnormal carbohydrate or abnormal fat metabolism. This conclusion is inferred largely from indirect evidence, since no specific data indicating primary disturbances in either neuronal or myelin metabolism in diabetics have yet been presented. The following points have bearing on the metabolic hypothesis:

POOR DIABETIC CONTROL. Diabetes mellitus is a metabolic disease and its complications might logically be suspected as having a metabolic basis. Rundles, Root and Kenny, Martin, and several others state that most of their patients with diabetic neuropathy had relatively long-standing diabetes of mild degree that was "poorly controlled." Ketosis or hypoglycemic shock was rare but the long-standing metabolic error was presumed to have induced an associated defect that predisposed to neuropathy. The observations of poor control are not universal, however. Neither Jordan nor Broch and Klövstad noted any difference in the antecedent diabetic control between patients with neuropathy and those without. It is our own impression that most diabetics with mild disease of long duration are disrespectful of extremely careful control so that the poor control may be a reflection of the mildness of illness and not a cause of complications.

Two additional observations are at variance with the poor control theory of pathogenesis. One is that neuropathy in a significant number of diabetics develops within two to six weeks *after* starting insulin therapy and the other is that the peripheral neuropathy may precede any other clinical manifestation of diabetes mellitus (Sullivan).

DISTURBED CARBOHYDRATE METABOLISM. Only fragmentary evidence indicates that hyperglycemia per se may result in neuropathy. Feldberg presented data indicating that high concentrations of glucose inhibited acetylcholine synthesis. Martin states that two patients who showed hyperglycemia as the result of acromegaly and hemochromatosis developed typical diabetic neuropathy. Clinical details on the patients were not given Neuropathy has not, to our knowledge, been reported in association with hyperglycemia induced by surgical pancreatectomy.

Furthermore, neuropathy is rare in the severe diabetic with intense hyperglycemia. No abnormality in pyruvate metabolism has been found consistently in diabetic neuropathy.

Abnormalities in the metabolism of both vitamin B_{12} and other vitamins have been found in diabetics (Stone and Chow; Field, *et al.*). These changes, however, are not particularly found in diabetics with neuropathy, and vitamin replacement as indicated above has no effect on the nerve lesions.

DISTURBED LIPID METABOLISM. An abnormal fat metabolism and an elevated serum lipid concentration are frequent in diabetics. Other diseases with elevated serum lipids (myxedema, nephrosis, familial hyperlipemia) carry no predisposition to neuropathy. If abnormal fat metabolism leads to demyelination in diabetes, it is peculiar that the process affects the peripheral nerves and roots almost uniquely. Phospholipids, cholesterol, and cerebrosides were all found to be subnormal in the nerves of diabetics by Jordan, Randall, and Bloor. However, the specimens were obtained from 18 patients over 52 years of age, 13 of whom had amputations and all but one of whom had deficient circulation. Randall later found that the nerves of patients with arteriosclerosis uncomplicated by diabetes showed the same changes in lipids as did the nerves of these diabetic patients.

Vascular Disease

The weight of evidence, both direct and indirect, positive and negative, suggests to ourselves that vascular disease may be the cause of diabetic neuropathy, but three principal observations are cited as evidence against this hypothesis. Each can be countered by other data:

1. Many clinical observations as well as skin temperature studies (Rundles, Martin) and oscillometric recordings (Martin) show unimpaired circulation in the extremities of patients with advanced neuropathy. (Such studies fail to *reflect the functional state of the small* arterioles and capillaries where vascular disease begins in diabetes (Ditzel). The absence of proximate arteriosclerosis does not necessarily rule out vascular disease of the nerves any more than it rules out vascular damage to the retina or kidney.)

2. Significant improvement or recovery is frequent in the neuropathy. (This course would only be difficult to reconcile with arteriosclerosis of the large arteries. *The peripheral nerves are served by a rich arteriolar* and capillary network from which collaterals can readily develop to compensate for localized areas of ischemia. Such improvement was actually occurring in Dreyfus' case cited below)

3. Widespread involvement of the autonomic neurones would not be

expected in occlusive vascular disease. (Confirmation of this point awaits appropriate pathology studies. Both prominent autonomic involvement and neural improvement are observed when the nerves are involved by periarteritis nodosa, however.)

Evidence supporting the concept of vascular disease causing diabetic neuropathy is both inferential and direct:

1. Such a cause is consistent with known pathologic changes taking place in the eye and the kidney in diabetics of long standing and requires no hypothesis of an undemonstrated error in neural metabolism.

2. The neuropathy bears a close relationship in incidence to other vascular lesions in diabetics. Retinopathy is found in from one third to 90 per cent (Rundles, Martin, Fagerberg) of patients with neuropathy. Neuropathy almost invariably accompanies diabetic nephropathy (Gilliland).

3. The available neuropathologic material (Woltman and Wilder; Fagerberg; Dreyfus, *et al.*) almost invariably describes thickening of the vasa nervorum in relationship to the nerve lesions of diabetes. In the few instances where such changes are undemonstrated, serial sections of the affected nerves have not been employed.

However, even if diabetic neuropathy proves to have a vascular pathogenesis, a number of important questions remain unanswered. If the ubiquity of peripheral vascular lesions found by Fagerberg is confirmed, this still leaves unanswered why the central nervous system is so rarely affected by the same process. Finally, although a vascular pathogenesis of the neuropathy would reduce the number of metabolic errors to investigate in diabetes mellitus, it still would leave unexplained the mechanism of the vascular lesion itself.

CLINICAL TYPES

Hyperglycemic Neuralgia

Diffuse muscular pain, particularly troublesome at night and localized more prominently in the lower extremities, frequently accompanies the early symptoms of untreated diabetes. Though paresthesias are occasionally reported in the feet, objective evidence of direct neural involvement is lacking. The symptoms subside promptly with insulin therapy and the consequent reduction of hyperglycemia. The mechanism of these painful symptoms is unknown.

Somatic Peripheral Neuropathy

Although many workers regard neuropathy as an entity, it has always seemed to this observer that diabetics were susceptible to two clinically

distinct types of peripheral somatic nerve disease. A certain number of elderly diabetics suffer disease of the peripheral nerves concomitantly with other manifestations of severe peripheral arteriosclerosis of the large vessels. The symptoms, findings, and prognosis of this neuropathy of arteriosclerosis differs in many ways from the more protean symptomatology of the neuropathy found in younger diabetics who have relatively well-preserved large arteries in the extremities. Thus in this section "arteriosclerotic peripheral neuropathy in diabetics" is treated separately from "diabetic neuropathy" since only the latter appears to be unique to the metabolic disease. Jordan made a similar distinction when he divided the diabetic neuropathies into a circulatory-degenerative type and a neuritic type.

PERIPHERAL NEUROPATHY ACCOMPANYING SEVERE ARTERIOSCLEROSIS IN DIABETICS. Reflecting the distribution of most severe vascular pathology, arteriosclerotic peripheral neuropathy affects principally the distal parts of the lower extremities. Sensory paresthesias are prominent, and are often accompanied by a burning sensation on the soles of the feet. Distal sensation is relatively symmetrically impaired, with loss of vibration sense being particularly prominent. A stockinglike distribution of hypalgesia and hypesthesia is frequent. While many of these patients suffer mild weakness, the motor impairment is seldom commensurate with the sensory loss.

Arteriosclerotic peripheral neuropathy is most often a problem of older diabetics and is accompanied by other signs of circulatory change. The patients suffer cold feet and circulatory stasis. The dry and poorly nourished skin readily becomes cracked and infected. Examination of the dorsalis pedis and posterior tibial arteries usually shows one or more of these to be pulseless. Lower extremity x-rays commonly reveal linear streaks of calcification along the course of the large arteries.

The treatment of arteriosclerotic peripheral neuropathy is tedious and the prognosis for recovery is poor. The feet should be kept warm and protected with heavy socks. Sudden excessive heating of the tissues is dangerous and special precautions are required to prevent burns or freezing since the reduced sensation impairs warnings of thermal injury. Priscoline, 25 to 50 mg., 3 to 4 times daily brings symptomatic relief to some patients, but 15 cc of whisky taken at 4-hour intervals is more likely to restore a sense of warmth to the extremities. Many of these patients develop indolent ulcers in the feet that are particularly refractory to treatment, and some still progress to require amputation despite antibiotics, sympathectomies, and local treatment of nonhealing soft tissue and bony lesions.

DIABETIC NEUROPATHY. In contrast to the neuropathy of arteriosclerosis, diabetic neuropathy is prone to be asymmetric in distribution, affects younger persons, is often accompanied by autonomic changes, and carries a better prognosis.

The neuropathy shows a particular predilection for the lower extremities although the upper extremities and trunk are frequently involved as well. The peroneal nerve is affected more than the posterior tibial, the femoral more than the sciatic, and the ulnar nerve more than the median or radial.

The most prominent and frequent symptom of diabetic neuropathy is pain, which often has an agonizingly deep and burning quality. The pain is peculiarly prone to develop at night and sometimes is exclusively nocturnal. Migrating muscle cramps and shooting dysesthesias often compound the discomfort. Characteristically the pain is poorly localized and distributed along neither the course of selected nerves nor nerve roots. Nerve tenderness is seldom prominent but cutaneous hyperalgesia may be so intense that the weight of the bed covers is intolerable. Many patients find that moving the extremities brings a measure of relief and some spend the entire night pacing their rooms.

Other sensory symptoms are nearly as frequent as pain. The patients may complain that the involved extremities feel "wooden" or that they feel as though they were walking on pillows. Tingling paresthesias are sometimes so intense that they preclude walking.

The objective sensory loss or hyperalgesia seldom parallels the intensity of sensory symptoms. Defects in vibratory sensation are frequent but serious positional loss is rare. Alterations in touch and pain sensation are often ill defined, difficult to reproduce exactly, and usually fail to conform fully to the known anatomic distribution of the major peripheral nerve trunks or nerve roots. The distribution of involvement is typically scattered so that motor and sensory findings in one member are seldom mirrored in the contralateral body part.

Motor disturbances are less prominent than the sensory symptoms and signs. The stretch reflexes are frequently diminished or lost, sometimes in muscle groups that are not symptomatically involved. Symptomatic weakness or paralysis is found in perhaps one third of the patients with sensory symptoms. Foot drop is the most frequent paralytic manifestation, but diffuse weakness and wasting of the legs, asymmetrical weakness of the arms or shoulders, and even isolated paralyses of the trunk muscles have all been encountered in our own patients. Occasionally, major motor symptoms develop in a neural distribution entirely separate from the most prominent sensory changes.

Cranial Nerve Neuropathies

Involvement of several cranial nerves, including the third, fifth, sixth, seventh, eighth, tenth, and twelfth, has occasionally been reported in diabetes mellitus. Aside from facial paralysis and external ophthalmoplegia, most of these are extremely rare and the findings may be coincidental.

Extraocular muscle paralysis occurs in about 1 per cent of diabetics (Walsh; Weinstein and Dolger). The complication is practically confined to patients over 50 years of age, most of whom have long-standing



Fig. 41-1. This 54-year-old diabetic developed sudden pain behind the right eye and diplopia. Examination showed equal pupils, a complete medial rectus paralysis, compensatory blepharospasm on the right, and no other abnormalities. The spinal fluid protein was 64 mg. per 100 ml. Recovery was complete in two months.

diabetes. Either the third or sixth nerve may be affected, but involvement of the fourth has not been reported. Generalized neuropathy is usually absent.

The onset of ocular paralysis often is accompanied by pain behind the eye and in the forehead on the same side. Third nerve paralyses seldom involve all the muscular branches of the nerve and the pupillary fibers are spared (Fig. 41-1). Both the third and sixth nerve are sometimes affected in the same eye and bilateral oculomotor paralysis is occasionally seen. The prognosis is good, most patients recovering within two to three months.

Diabetic ophthalmoplegia appears to be peripheral in origin, the lack of associated signs of intramedullary disease is consistent with this view. The single well-studied autopsied case of Dreyfus, Wakim and Adams showed a fusiform swelling of the retro-orbital portion of the oculo-

motor nerve. There was destruction of some of the myelin sheaths and axis cylinders in the center of the nerve and an increase in the neural connective tissue (Fig. 41-2). Only retrograde changes were found in the oculomotor nuclei. Thickening of the vasa nervorum was observed (Fig. 41-3). Although serial sections failed to show a complete occlusion



FIG. 41-2. Low-power cross section of involved part of left oculomotor nerve from case of diabetic ophthalmoplegia. The central demyelination is reflected by pallor and the more normal periphery stains darkly. (Heidenham myelin stain magnified × 37.) (From Dreyfus *et al.*, with permission of the *A.M.A. Arch. Neurol. & Psychiat.*)

of an artery or vein, the authors concluded that the lesion was compatible with an incomplete ischemic neuropathy.

Involvement of the Autonomic Nervous System

Impaired autonomic function frequently accompanies the somatic neuropathy of diabetes. Pupillary abnormalities, orthostatic hypotension, disorders of sweating and peripheral circulation, urinary retention, sexual impotence, neuropathic joints, and paroxysmal, crisislike,

abdominal pains sometimes produce such a close similarity to parenchymatous neurosyphilis that the clinical picture has been called diabetic pseudotabes. Central lesions in the hypothalamic autonomic centers or in descending autonomic pathways have not been found in such patients. Although the available pathologic material is limited, the best evidence favors a peripheral neural basis for these autonomic abnormalities of diabetics.

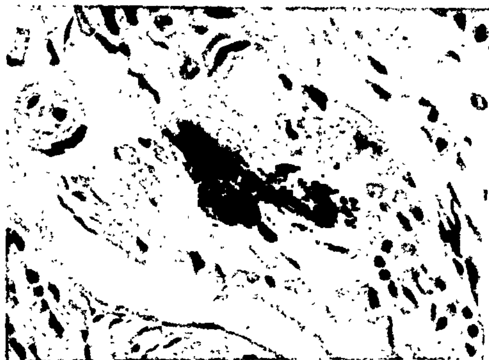


FIG. 41-3. Eosinophilic fibrinoid material deposited adjacent to a small artery in the nerve illustrated in Fig. 41-2. (Hematoxylin and eosin magnified × 450) (From Dreyfus *et al.*, with permission of the publisher.)

PUPILLARY CHANGES. Abnormal pupils are reported in from 9 (Martin) to 25 per cent (Rundles) of patients with diabetic neuropathy. Miosis with pupillary inequality is the most frequent finding. True Argyll-Robertson pupils showing miosis, inequality, irregularity, a reaction to accommodation but not to light, and a sluggish reaction to mydriatics have been reported by many workers. These changes are asymptomatic and of theoretical interest in that they provide inferential evidence for a peripheral genesis of the Argyll Robertson pupil.

PERIPHERAL AUTONOMIC AND CIRCULATORY CHANGES. Ankle edema, defective or absent sweating, loss of vasomotor and pilomotor control, and

intolerance to extremes of temperature are found in one third or more of patients with diabetic neuropathy. Objective tests have repeatedly demonstrated sweating loss or a failure of reflex vasoconstriction and vasodilatation in the lower extremities when the trunk was cooled or warmed (Rundles, Martin). Rundles first emphasized that patients with diabetic neuropathy also may have symptomatic orthostatic hypotension owing to a failure of reflex vasoconstriction in the legs. Reflex orthostatic cardiac acceleration is unimpaired.

Trophic skin changes are closely related to the peripheral autonomic disease. Atrophy of the skin of the feet is common as is dryness and fissuring in the same areas accompanied by trophic changes in the nails. Perforating ulcers of the feet, similar to those of tabes dorsalis, are sometimes encountered despite the apparent absence of any large artery disease. These painless lesions may penetrate to the underlying bone unless weight bearing is promptly interrupted.

NEUROPATHIC JOINT CHANGES. Neurogenic osteoarthropathy in diabetes usually involves the intrinsic bones of the feet, in contrast to syphilitic Charcot joints, which are usually more proximal. The pathogenesis of the Charcot joint is not altogether clear, but there is much to suggest that damaged autonomic control is at least as important as is interruption of afferent pain pathways. Occasionally, the arthopathy of diabetic neuropathy may develop acutely with sudden swelling, warmth, and redness. More frequently the development is indolent, however, and discovered only because of increasing difficulty in walking. The prognosis for recovery is poor once advanced joint changes have developed. Therefore, incipient abnormalities should be treated by interrupting weight-bearing until the other signs of neuropathy improve. Parsons and Norton suggest that sympathectomy may halt the progression of joint changes but extensive observations are lacking on this point.

GENITOURINARY CHANGES. Temporary impotence is fairly frequent in diabetic males during the untreated phase of their disease or following episodes of infection, diabetic acidosis, and insulin hypoglycemia. More severe and irreversible impotence is a prominent accompanying symptom of men with diabetic neuropathy. Rubin and Babbott found that 49 of their 198 diabetic men became impotent before age 50. Exact figures from randomly selected diabetic men in other diabetic clinics are not available. However, Rundles found impotence in 27.5 per cent of his men with diabetic neuropathy and Martin noted that 37 per cent of men under age 50 with diabetic neuropathy were impotent. Several things suggest that the impotence is neurogenic: The disturbances in gaining and maintaining erections usually precede loss of libido; normal spermatozoa have been obtained from impotent diabetic men

(Oakley); Martin found absent bulbocavernosus reflexes in all cases of diabetic impotence and noted that one third of his 38 cases had disturbances of micturition as well.

Abnormalities in urinary bladder and sphincter function develop in approximately 10 per cent of patients with diabetic neuropathy, and are more frequent in men than in women. Urinary hesitancy, slowness of urination, dribbling incontinence, or intermittent retention are frequent complaints. The outstanding changes on cystometric and cystoscopic examinations are a dilated, trabeculated bladder with a markedly impaired filling sensation and with weak detrusor contractions. In all probability, impaired filling sensation and a loss of afferent bladder stretch impulses are the primary disturbances.

Once urinary retention has developed, the prognosis for spontaneous recovery of bladder function is poor and the large residual urine often leads to infection. Treatment should be directed toward decompressing the dilated bladder by intermittent drainage, thus allowing the smooth muscle tonus to be restored. Once the majority of infection has been cleared, Urecholine can be employed to facilitate micturition. To be effective, the drug is best given at first parenterally at regular intervals for several days and then orally to supplement voluntary efforts at bladder emptying. Patients with refractory bladder atony and continued high residual urines should be considered for bladder neck resection since infection almost certainly will persist until the residual urine is eliminated.

GASTROINTESTINAL CHANGES. Serious disturbances in gastrointestinal motility plague a large percentage of patients with diabetic neuropathy. Distressingly persistent constipation is the most frequent abnormality and leads to the chronic use of laxatives and enemas for relief. Diarrhea is only slightly less frequent than constipation and in many instances alternates with it. The diarrhea is particularly disturbing since it has a peculiar tendency to develop at night or in the early morning hours, at which time fecal incontinence often results. With severe diarrhea abdominal cramping and even nausea and vomiting may be so pronounced as to be reminiscent of the gastric crises of tabes dorsalis.

The mechanism of these gastrointestinal changes in diabetic neuropathy is speculative. Roentgenographic examinations (Hodges, *et al.*, Martin) are generally less revealing than one might anticipate. A delay in gastric emptying time is fairly frequent and the barium may show prolongation of its passage through the small intestine with segmentation in that organ. The stools are normal and gastric anacidity is the exception. Rundles mentions finding "degenerative changes in the nerve trunks of the esophageal plexus and about the celiac ganglia" in two patients with gastrointestinal symptoms who died.

Severe gastrointestinal symptoms subside or improve with time and general treatment in most patients with diabetic neuropathy. However, recurrences are frequent and the roentgenographic abnormalities are persistent (Hodges, *et al.*).

SPINAL FLUID

Approximately 70 per cent of patients with diabetic neuropathy show an abnormally elevated spinal fluid protein content (Ives). The protein content usually ranges between 50 and 100 mg. per 100 cc. Occasionally, however, values as high as 100 to 200 mg. per 100 cc. or more are found (Table 41-2). The pathogenesis of the abnormality is apparently

TABLE 41-2. THE SPINAL FLUID PROTEIN IN DIABETIC NEUROPATHY

Author	*Total cases studied*	*Number with normal protein*	*Number with mild elevation (approx. 50–75 mg. per cent)*	*Number with moderate elevation (approx. 75–120 mg. per cent)*	*Number with marked elevation (over 100–120 mg. per cent)*
Ives	39	12	12	9	6
Jordan	40	18	7	15	
Martin	26	16	4	2	4
Root and Kenny	157	11	43	53	17
Rundles	39	10	5	11	13
Totals	301 (100%)	33%	24%	30%	13%

related to the neurological lesion since Madonick and Margolis found normal spinal fluids in 80 diabetics who were free from neurologic complications. However, there is little relationship between the absolute protein level and the severity of the neuropathy.

Knowledge of protein formation and absorption in the spinal fluid is too meager to derive an accurate explanation for the changes in diabetes. The length of disease, the use of insulin, the quality of treatment, and the age of the patient all have relatively little relationship to the spinal fluid findings. It is possible that the elevated protein is nonspecific and reflects no more than the frequent involvement of nerve roots in the neuropathic process. The vascular permeability of subarachnoid capillaries has not been studied in diabetics.

TREATMENT AND PROGNOSIS

The principal treatment of diabetic neuropathy is the treatment of the diabetes itself. Almost all workers in the field claim that the

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TREATMENT AND PROGNOSIS

The principal treatment of diabetic neuropathy is the treatment of the diabetes itself. Almost all workers in the field claim that the

neuropathy subsides *pari passu* with the institution and maintenance of good diabetic regulation. Control data to confirm this statement are lacking since few willingly permit their diabetics to continue grossly unregulated.

The rate of recovery in diabetic neuropathy is unpredictable. Some patients get worse after insulin is instituted, some improve strikingly in a matter of weeks and some show only gradual improvement over a period of many months. Fortunately, severe pain is usually the first symptom to subside and improvement in motor function is almost universal. However, even patients with marked improvement seldom completely lose their sensory symptoms and a permanent loss of the distal stretch reflexes in the legs is frequent.

Symptomatic measures are required to bring relief for diabetic neuropathy in the acute stage of the disease. The pain sometimes requires codeine but more powerful narcotics are contraindicated because of the long duration of the disease. Cradles are helpful to keep bed covers off hyperalgesic areas, and footboards or splints should be employed to prevent Achilles tendon contractures. If physiotherapy, standing, and walking are started as soon as the pain subsides, disuse changes in skin, muscle, and bone will be minimized.

SPINAL CORD DISEASE

Diabetic myelopathy is rare and has been doubted as an entity by some who feel that all the described spinal changes are secondary to peripheral nerve or nerve root disease. Certainly, anterior horn cell degeneration can be explained on a retrograde basis and much of the posterior column degeneration is secondary to dorsal root disease. However, Griggs and Olsen have described, from the literature and from their own material, cases which clearly showed primary spinal cord lesions. Clinically, Babinski signs were found as well as other clinical abnormalities that could have been either peripheral or central in origin. Pathologically, the spinal cord showed segmentally localized degeneration in the posterior columns with localized necrosis and degenerative changes in the lateral columns as well. There was prominent thickening and hyalinization of the arterioles in the involved areas.

EFFECTS OF DIABETES ON THE BRAIN

Neither clinical nor pathological studies indicate that any specific brain lesions follow diabetes mellitus. Cerebral infarcts resulting from arteriosclerotic vascular disease are more frequent in diabetics than in the nondiabetic population of equivalent age. Approximately one half

of diabetic patients with cerebral infarction shown an elevated spinal fluid protein (Ives) but otherwise the diabetic state does not modify the clinical neurological picture.

Despite the lack of clinical neurological changes, electroencephalographic studies show a much higher incidence of abnormalities among diabetics than among the normal population (Greenblatt, Murray, and Root, Izzo, Schuster, and Engel). Both abnormally slow and abnormally fast activity have been described. If one accepts slow activity as a more certain indication of abnormality, the electroencephalographic changes are particularly prone to occur in diabetics with a history of frequent severe insulin reactions. However, less striking, minor deviations from normal are encountered both in mild diabetics with no history of insulin reactions and in the nondiabetic blood relatives of diabetics (Fabrykant). Except in the patients who have suffered recurrent hypoglycemia, the significance of these cerebral electrical changes is unclear and, in part, still controversial. It has been suggested by some that genetic factors, linked to the metabolic error, may account for the electroencephalographic changes. Whatever the cause, however, the electrographic alterations have no symptomatic counterpart and require no treatment.

Depression is frequent in diabetic patients with severe neuropathy. This is hardly a surprising accompaniment to a severely painful and incapacitating disease and the psychic changes appear to be nonspecific. Mental deterioration following hypoglycemic reactions and more chronic personality changes associated with mild, unrecognized hypoglycemia are mentioned in Chapter 48. Other than these emotional reactions to severe illness and the effects of hypoglycemia, there are no specific intellectual or personality changes that can be attributed to diabetes (Kabany, Danowski, and Moser).

Diabetic acidosis produces a profound disturbance in brain function and, when severe, leads to reduced cerebral blood flow and oxygen consumption. The cellular mechanisms producing the coma are unknown, but residual neurological defects are practically unheard of if the metabolic disease is treated effectively.

NEUROLOGIC COMPLICATIONS OF INSULIN THERAPY

HYPOGLYCEMIA

The protean symptomatology and potentially dangerous effects on the brain of hypoglycemia are discussed in detail in Chapter 48. The frequency of hypoglycemic symptoms owing to insulin therapy is speculative, but on the Neurological Service of the King County Hos-

TABLE 41-3 TRANSIENT NEUROLOGICAL DISORDERS CAUSED BY HYPOGLYCEMIA*

Patient	Signs and symptoms	Original diagnosis	Blood sugar	Daily insulin dose: Before attack	Daily insulin dose: After	Time required to recover to normal after care given
1. R. Q. 12 ♂	Recurrent generalized convulsions	Epilepsy	32	10 u NPH	15 u NPH	Immediate
2. E L. 37 ♂	Excessive drowsiness and somnolence while driving, at work, etc.	Narcolepsy	18	10 u NPH	10 u NPH	8 hours
3. F S 37 ♂	Left hemiparesis (conscious)	Cerebral infarction	31	35 u PZI	35 u PZI	Immediate
4. G. H. 39 ♂	Recurrent manic attacks, assaulted family	Functional psychosis	9	15 u Lente	same	6 hours
5 M C. 39 ♀	Status epilepticus with CSF pleocytosis	Acute meningitis	21	30 u PZI	unknown	Immediate
6. R. N. 42 ♂	Reckless driving, dysarthria, ataxia, amnesia	Acute intoxication	28	20 u Reg. 10 u NPH	unknown	Immediate
7. R. B. 44 ♂	Stuporous, irrational, no initial response to glucose	Acute psychotic reaction	58	20 u Lente 10 u Reg.	DBB 75 mg. q.i.d.	6 hours
8. S. B. 52 ♀	Extensor hypertonus, bilateral Babinski, coma	Basilar artery thrombosis	29	60 u NPH	30 u NPH	2 hours
9. C. P. 64 ♂	Coma of unknown origin	Basilar artery thrombosis	33	10 u NPH	36 u NPH	Immediate
10. J B 65 ♀	Deep coma with recurrent tetanic spasms	Basilar artery thrombosis	16	40 u NPH	DBI 25 mg. q.i.d.	7 days

* The hypoglycemic origin of symptoms was at first unrecognized and led to the indicated diagnoses. Cases 2, 6, 9, and 10 were not known to be diabetic until the hypoglycemia had been treated. All these patients have remained neurologically well following adjustment of insulin dosage or diet or both. Note that hours or days may be required for recovery when hypoglycemia is profound. Cases 5 and 6 were treated as emergencies only and final diabetic regulation was carried out elsewhere. DBI = phenethylbiguanide, DBB = amylbiguanide.

pital at least 10 new patients are seen annually with serious neurologic problems resulting from insulin-induced hypoglycemia. Most of these subjects have taken a regularly prescribed dose of the drug and are either chronically receiving too much insulin or have temporarily interrupted eating because of alcoholism, intercurrent disease, or the press of other affairs. Table 41-3 lists a series of such patients in whom incorrect neurologic diagnoses by physicians preceded the recognition and treatment of hypoglycemia. It should be emphasized that when hypoglycemia has been profound or protracted, amelioration of neurologic symptoms may require hours or days. A failure to appreciate this phenomenon of delayed recovery has been the factor that led to erroneous diagnoses of primary neurologic diseases in a significant number of the patients we have seen. Such diagnostic errors can be avoided if blood is always drawn for sugar determination before glucose is given to treat a suspected insulin reaction.

HYPERINSULIN NEURONOPATHY

Distal paresthesias in the extremities associated with muscular atrophy and weakness are a rare complication of hyperinsulinism. Mulder, *et al.*, reported 20 cases, 18 of whom had pancreatic adenomas or adenocarcinomas. However, distal paresthesias have also been noted after coma owing to exogenous insulin (Ziegler) although associated muscular weakness and atrophy were noted in only two of the 13 subjects.

Part of the reason for the apparent rarity of involvement of peripheral neural structures after insulin coma may be that the more threatening central nervous system effects of hypoglycemia overshadow the peripheral symptoms. Anterior horn cell damage in the spinal cord has been observed in both humans and experimental animals following insulin coma but pathological studies of peripheral nerves have not been carried out.

Clinically, symptoms of peripheral neural damage usually follow severe episodes of hypoglycemia. There is tingling or burning in the feet and hands, which is only occasionally accompanied by objective sensory loss. Muscular atrophy, though usually confined to hands and feet, sometimes progresses to involve the trunk as well. The muscle weakness is observed particularly with dorsiflexion of the feet and fine movements of the hands. The deep reflexes are characteristically hyperactive except for the Achilles reflex, which is reduced or absent. Muscle fasciculations have been noted only occasionally. Progression of the disturbance is halted if recurrences of hypoglycemia are prevented.

In a diabetic with recurrent hypoglycemic crises, differentiation be-

tween hyperinsulin neuronopathy and diabetic neuropathy may be difficult. The former is more symmetrical, produces more motor involvement, and carries fewer objective sensory signs than the latter. However, these peripheral complications provide additional reasons for stringent efforts to avoid hypoglycemic attacks in patients receiving insulin.

SUMMARY

Peripheral neuropathy is the most frequent and perhaps the only specific neurologic complication of diabetes mellitus. The exact etiology of diabetic neuropathy is unknown, and opinions vary about its relationship to diabetic control with insulin. Critical biochemical and pathologic observations are still fragmentary, but it appears that the neuropathy may have a vascular genesis similar to the genesis of diabetic retinopathy and nephropathy.

Both somatic and autonomic nerve fibers are affected in diabetic neuropathy. Sensory impairment is more frequent than is motor disability and pain is the most distressing symptom. The autonomic lesions produce prominent pupillary, circulatory, joint, genitourinary, and gastrointestinal abnormalities. Diarrhea and impotence are particularly prevalent and troublesome symptoms.

Time, symptomatic care, and general diabetic regulation must be relied upon in treating diabetic neuropathy. Most patients with the disease do relatively well and show considerable improvement, although most have residual, permanent neurologic defects with reflex loss, and at least minimal distal sensory loss is frequently encountered.

While not a direct effect of the disease, insulin-induced hypoglycemia is an additional source of potential danger to the nervous system of diabetics. Electroencephalographic abnormalities are frequent among diabetics receiving insulin and symptoms of peripheral as well as central nervous system damage may follow severe insulin coma.

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Chapter 42

DERMATOLOGIC LESIONS AND DISEASES ASSOCIATED WITH DIABETES

Thomas B. Fitzpatrick

A heterogeneous group of dermatologic lesions and diseases is associated with diabetes mellitus. While none of these can be said to be pathognomonic of diabetes mellitus, their association is frequent enough so that the clinician alerted to these few dermatologic signs may suspect diabetes mellitus (Fig. 42-1).

The important dermatologic lesions and diseases associated with diabetes will be discussed in the following sequence:

- Carbohydrate metabolism of the skin (normal and in diabetics)
- Cutaneous infections
  - Pyodermas
  - Candidiasis (moniliasis)
  - Dermatophytosis
- Lipidoses
  - Xanthoma diabeticorum
- Miscellaneous
  - Necrobiosis lipoidica (diabeticorum)
  - Insulin lipodystrophy

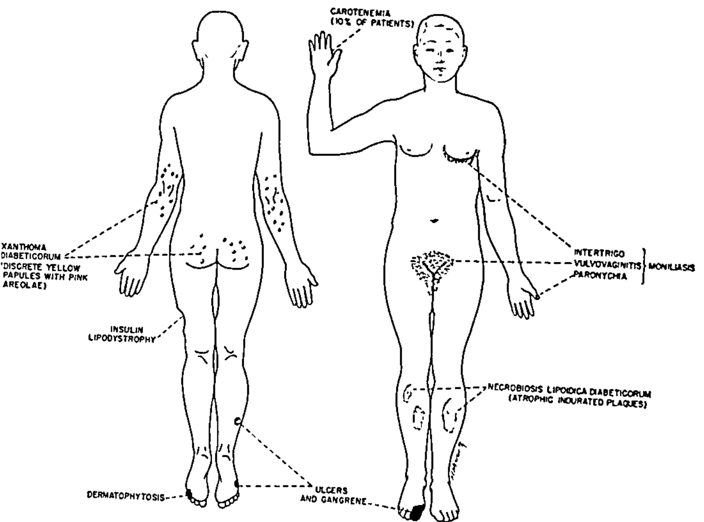


FIG. 42-1. Dermatologic disorders associated with diabetes mellitus.

Pruritus (localized and generalized)
Carotenemia

A few other conditions have been linked with diabetes mellitus, but their association has not been frequent enough to be significant. These include xanthelasma, Dupuytren's contracture, psoriasis, porphyria, and gout.

CARBOHYDRATE METABOLISM OF THE SKIN

Normal Metabolism

The skin is perhaps unique in that it does not contain a known system for metabolism of tricarboxylic acids in the cyclic manner of Krebs or a mechanism for carbohydrate synthesis from pyruvate. In addition, there is a slow or possibly no synthesis of cocarboxylase from thiamin and phosphoric acid. This has led to the speculation that these inherent enzyme deficiencies may account for the early appearance of cutaneous changes in nutritional deficiencies especially of the B Factors. The skin contains glycogen but it is apparently not available for metabolic processes.

Much of the research on the carbohydrate metabolism of the skin must be repeated on the epidermis and dermis separately since there are now available several methods for splitting skin into its two major parts. In this manner it may be possible to detect enzymes that are involved in the Krebs cycle which may be present but in an inhibited state as suggested by Lerner (1954).

Parenteral or oral administration of glucose causes a rise in *skin* glucose, and there is a delay in the return to normal fasting levels. Whether this delay is a matter of simple diffusion processes or is an actual storage of glucose by means of adsorption on extracellular elements, such as connective tissue ground substance that cannot metabolize glucose, is not known. This adsorption might explain the "lag" in the return to normal of cutaneous sugar in hyperglycemia and would account for the higher content of glucose in well-fed animals that have been fasted.

Diabetes Mellitus

The investigations of Urbach in the early 1940's resulted in the hypothesis that there existed in patients with diabetes mellitus, a "skin diabetes," as detected by a "skin glucose tolerance test." The latter revealed a diabetic type of curve with a sharp rise of glucose, a delayed maximum after 3 hours, and a delayed return to normal values amount-

ing to approximately one hour longer than the return of the blood glucose levels and very high fasting skin glucose level and abnormal skin glucose tolerance curves in patients with a normal glucose tolerance test. This led to the belief that the skin in diabetics was susceptible to infections, intertriginous eczemas, etc., because of high content of glucose in the skin. (See later discussion of infections.) These observations by Urbach have not yet been confirmed.

INFECTIONS OF THE SKIN IN DIABETES MELLITUS

Early evidence by Pillsbury and Sternberg indicated that experimental pyogenic infections were more severe in dogs on a high carbohydrate diet than in those either on a low carbohydrate, high fat diet or fasting. A parallel effect in man has not been clearly established. The notion that the increased glucose content of the skin in diabetics provides a more suitable culture medium for bacteria and fungi has not been readily accepted in view of several inconsistent findings. (1) In earlier reports patients with severe diabetes were shown to have a normal resistance to infection. (2) In a very early investigation in Germany, Handmann showed that staphylococci in vitro grew no better on blood containing 0.5 to 1.0 per cent glucose than on normal blood and that the addition of glucose to blood within the limits found in diabetes did not alter the bactericidal effect.

The possible effect of dehydration on the susceptibility to experimental staphylococcic infection is suggested by the studies of Pillsbury and Kulchar (1935) since their rabbits showed a marked increase in the severity of staphylococcic infections when given large amounts of parenteral glucose or sodium chloride. This is consonant with the clinical impression that a lowered resistance to infections is present in patients with prolonged polyuria, glycosuria, dehydration, and acidosis.

It should be emphasized that diabetics whose carbohydrate metabolism is restored to normal by insulin appear to have a normal resistance to infection. Studies are needed on the quantity and type of globulins present in "well-controlled" and "poorly controlled" diabetics.

Pyodermas

The study of Greenwood (1927) in 500 diabetics indicated double the number of furuncles and carbuncles in diabetics as compared with the nondiabetic. However, diabeti[illegible] ts wit[illegible] les and carbuncles are more often hospitali[illegible] ndia[illegible] ts with the same problem. Williams (1942 [illegible] are [illegible] inion that pyodermas are no more common [illegible] tha[illegible] 'cs. While

there may be disagreement on the incidence of pyodermas in diabetics, there is universal agreement that pyodermas, however slight, demand prompt medical and surgical treatment. Even mild infections may alter the insulin requirement and result in acidosis. The use of antibiotics has dramatically changed the problem of pyodermas. However, with the advent of resistant strains of staphylococci (micrococci) and the problem of agammaglobulinemia, the clinician treating a pyoderma in a diabetic must determine the sensitivity of the organism, select the proper antibiotic, and administer γ-globulin, if indicated. Even if the organism is sensitive to penicillin, the alarmingly high and sometimes fatal allergic reactions to penicillin have markedly limited its use.

Candidiasis (Moniliasis)

It is almost axiomatic that in nonpregnant females pruritus and inflammation of the vulva is caused by *Candida albicans* until proved otherwise. Furthermore, the presence of candidiasis of the vulva or any other area of the skin or mucous membrane demands a search for diabetes mellitus as a precipitating factor.

The concept of "diabetic vulvitis" was advanced by Hesseltine in 1933 and its etiology established as *Candida albicans* by the same author in 1938. He noted a disappearance of the vulvitis with candicidal therapy but not with control of the diabetes and the disappearance of the glycosuria. In the presence of *Candida albicans*, the application of glucose in a powder or aqueous solution was associated with the development of a candidiasis, or an exacerbation of monilial vulvitis. Candidiasis in the diabetic in one series (Greenwood, 1927) was predominantly limited to the vulvar mucous membranes with occasional extension to the skin, while in the nondiabetics surveyed, the involvement was mainly intertriginous, affecting the submammary, inguinal, and axillary regions. The possible growth-promoting property of glucose for *Candida albicans* is therefore suggested by (1) the exacerbations following application of glucose solutions to the patient with a quiescent monilial infection of the vulva and (2) the localization of the candidiasis in the diabetic to the vulva, which is bathed in glucose-loaded urine in the uncontrolled diabetic. There have been reports of diabetics with generalized cutaneous and systemic candidiasis, particularly in children.

Pillsbury, Shelley, and Kligman (1956) have emphasized the low virulence of *Candida albicans*. The organism occurs as normal habitant of the normal and abnormal mucous membrane and the abnormal skin surface. In addition to diabetes mellitus as a primary factor in the development of candidiasis, other predisposing factors in the diabetic or

nondiabetic include obesity, chronic malnutrition, and dehydration (moniliasis of the esophageal lining is a commonplace finding at necropsy in patients dying of chronic emaciating disease such as malignancy), and possibly antibiotic therapy. The role of antibiotics in the pathogenesis of candidiasis is not clear; the antibiotics are apparently not growth-promoting factors for *Candida albicans* but the imbalance of the normal flora of the gastrointestinal tract as a result of the action



Fig. 42-2. Vulvovaginitis in a diabetic, caused by *Candida albicans*. Note the satellite pustules in the crural areas.

of antibiotics may very well be a factor in the development of clinical candidiasis.

CLINICAL FEATURES. Candidiasis of the mucous membrane is the most frequent type seen in diabetes. Although the oral mucous membrane (thrush, perlèche) may rarely be involved, by far the most frequent localization is the vulva and vaginal mucosa. Vulvovaginitis is accompanied by severe and intractable pruritus. The vulva is slightly or markedly swollen, tender, and has a red-blue basic color and a thin grayish surface (Fig. 42-2). Abrasions and excoriations are present. The hymenal ring is most frequently involved, with extension to the moist

surfaces. Not uncommonly the eruption extends upward to the mons veneris, outward to the crural fold, and posteriorly to encircle the anus. Small, easily removed, white plaques or cheesy material is seen on the surface.

The clinical picture of vulvovaginitis may vary considerably and, in all patients with vulvar pruritus, a specimen should be obtained for culture. Direct examination of material removed from the area is unreliable and culture of the organism is the only positive method of establishing the diagnosis of candidiasis. Nickerson's medium offers a quick, reliable method of identification, since the chlamydospores are easily identified—being differentially stained by a dye incorporated in the medium.

Other forms of cutaneous candidiasis may occur in diabetics including onychonychia and paronychia, or more rarely a generalized mucocutaneous involvement including stomatitis, glossitis, papules, pustules, and eczematous changes especially localized in the inframammary, periumbilical and axillary areas. There have been a few reports of systemic moniliasis in diabetics with endocarditis, pulmonary and bronchopulmonary lesions.

TREATMENT. Nystatin (Mycostatin), a candicidal antibiotic, is available in almost all forms for treatment of moniliasis. Vaginal tablets and powder are very effective for the treatment of vulvovaginitis. Oral tablets and flavored powder for dilution are available for adults and children respectively. It is curious that although the oral nystatin is apparently not absorbed and blood levels are not obtainable, the preparation is often effective in generalized cutaneous lesions. Nystatin inhibits the growth of *Candida albicans* in the gastrointestinal tract. The oral dosages of nystatin are 500,000 units three times daily for adults and 100,000 units three times daily for children.

Dermatophytosis: A Predisposing Factor in Gangrene

Gangrene of the lower extremities is one of the most serious complications in the diabetic. Second only to arteriosclerosis obliterans in the diabetic as a cause of gangrene is the secondary infection resulting from dermatophytosis of the feet.

The incidence of superficial mycoses particularly those involving the feet, hands, and nails (the dermatophytoses) is probably no higher in diabetics than nondiabetics. In Greenwood and Rockwood's study of 100 mostly controlled diabetics, 70 per cent were found to have dermatophytosis of the interdigital areas of the feet. There was no direct relationship between the incidence or severity of involvement and the level of hyperglycemia. Although reliable statistical data are not

available, the incidence of dermatophytosis of the feet in the general population is estimated at between 60 and 80 per cent. However, dermatophytosis of the interdigital spaces in the diabetic is a major problem because the epidermal fissures and erosions are portals of entry for β-hemolytic streptococci and staphylococci, leading to cellulitis, and "infectious" gangrene of the digits. Other predisposing factors besides dermatophytosis that lead to gangrene in the diabetic are impaired arterial circulation (11 times as frequent in diabetics as nondiabetics), diabetic neuritis, corns, calluses, and trivial foot injuries resulting in cuts or abrasions that become secondarily infected.

CLINICAL FEATURES. *The clinical picture of dermatophytosis of the feet is often diagnostic.* The chronic type consists of scaling, hyperkeratotic areas without vesicles or pustules. The sites of predilection are the webs of the toes (particularly between the 4th and 5th toes), the heels, sides, and soles, often distributed in an area covered by a ballet shoe. *In the toe webs the areas appear white, macerated, and soggy, with* ominous fissures. When the superficial white membrane is removed a red, ham-colored base is seen. The more acute and subacute types are characterized by vesicles and pustules involving the entire sole and spreading to the dorsum of the feet, or localized in coin-sized areas. An eczematous process may be superimposed on the subacute and acute type. This is characterized by minute vesiculation, erythema, crusts, and marked pruritus. Ulcers and erosions may develop from ruptured vesicles. In all three types (acute, subacute, and chronic) lymphangitis, cellulitis, and lymphadenitis may develop suddenly.

Dermatophytosis of the feet is more common in males living in tropical or subtropical areas; during the summer it is common in northern latitudes.

TREATMENT OF DERMATOPHYTOSIS AND CARE OF THE FEET IN THE DIABETIC. It is doubtful whether a "cure" of dermatophytosis is ever achieved. There is a marked individual variation in susceptibility and resistance to dermatophytosis. Some persons are resistant to the development of clinical dermatophytosis regardless of the magnitude or frequency of exposure. The causes of the lowered resistance to dermatophytosis in some individuals have not been determined. This is a much neglected area of research. Metabolic studies of patients with superficial mycoses should be made to determine whether persons susceptible to dermatomycoses have altered metabolic pathways.

Pending the development of systemic methods of treatment of dermatophytoses, certain fairly effective topical antifungal agents are available. Prophylaxis should be used in all diabetics with dermatomycoses of the feet. This includes the development of foot conscious-

ness in the diabetic: (1) daily bathing with a change of socks; (2) the use of antifungal powders containing low-molecular fatty acids (Sopronol and Desenex); (3) almost constant use of well-aerated sandals (dermatophytosis of the feet is rare in native Japanese); (4) prompt management of small clusters of vesicles and erosions.

The use of strong, irritating antifungal ointments in the diabetic is to be condemned. This includes the old war horse, Whitfield's ointment. In the acute and subacute phases, wet soaks with weak (1:9000) potassium permanganate and solutions and powders of fatty acids (e.g., Desenex) should be used. In chronic, hyperkeratotic dermatophytosis the treatment is usually unsatisfactory. In some patients, a new antifungal solution containing low-molecular fatty acids and copper (Verdefam) has been temporarily effective. The diabetic patient should be warned not to obtain various proprietary "salves" and liquids from druggists for the treatment of "athlete's" feet.

The use of chiropodists and foot clinics in many centers for the treatment of diabetics has been helpful in the early detection and management of corns, calluses, ill-fitting shoes, dermatomycosis, etc.

XANTHOMA DIABETICORUM

DEFINITION. Xanthoma diabeticorum is the term used to describe the scattered papules and nodules occurring rarely in some diabetics who have moderate or marked hyperlipemia. The designation *xanthoma eruptivum* sometimes used to describe this disorder has no special meaning and should probably be dropped.

PATHOGENESIS. A relative or absolute deficiency of insulin results in a decrease in carbohydrate utilization. To provide energy the body then mobilizes the fat depots and there is a phagocytosis of lipids (predominately neutral fat) by histiocytes and phagocytic cells in the liver, spleen, retina, and skin. The hyperlipemia present in glycogen-storage disease, lipoid nephrosis, and idiopathic hyperlipemia may also be associated with similar skin lesions and the term *xanthoma diabeticorum* must be considered as a secondary hyperlipemic xanthomatosis with no special or unique features except the presence of diabetes.

The papules and nodules of xanthoma diabeticorum consist of nests of histiocytes containing lipid (foam cells) similar to the histologic pattern present in primary hypercholesteremic xanthomatosis. In the chemical studies of excised lesions, Wile, *et al.*, found that cholesterol occurs to the extent of only 16 per cent of the lipids present.

Droplets of neutral fat accumulating in the intervascular spaces of the retina produce a pale grayish-pink fundus with white blood vessels.

This is called *lipemia retinalia* and may coexist with xanthoma diabeticorum.

CLINICAL FEATURES. The disorder appears to be more common in men. The lesions are bilateral and are characteristically present on the buttocks, extensor surfaces, palms, and soles. They occur as *firm, discrete* papules and nodules (Fig. 42-3). The color of the lesions is ordinarily a blend of pink and yellow, or a brilliant yellow with a pink halo. The



FIG 42-3. Xanthoma diabeticorum

lesions often look like pustules, but they are solid when incised. There may be marked pruritus with excoriations.

The spleen and liver may be enlarged as the result of accumulation of fat. Liver function studies, however, are not abnormal.

The diabetes mellitus may be mild or severe, although the majority of reported cases have occurred in severe diabetes.

LABORATORY FINDINGS. The serum is lactescent, owing to the presence of high concentrations of neutral fats and relatively low concentration of phospholipids. The latter have been said to act as emulsifiers and when proportionately reduced in relation to the other serum lipids the serum is milky.

DIAGNOSIS. The rather acute onset and distribution pattern of the characteristic pruritic yellow papules surrounded by a pink halo provide a relatively typical clinical picture. The presence of diabetes mellitus and hyperlipemia confirms the diagnosis. The lesions of primary hypercholesteremic xanthomatosis may have a similar distribution pattern but appear insidiously, are fewer in number, and the nodules are usually larger in size. In addition, the serum in primary hypercholesteremic xanthomatosis is not milky but clear.

COURSE. The lesions disappear with the treatment of the diabetes mellitus and the restitution of normal carbohydrate utilization, and the reduction of serum lipids to normal. Dietary fat restriction is not necessary.

NECROBIOSIS LIPOIDICA

DEFINITION AND CLASSIFICATION. A peculiar dermatologic disorder consisting of sharply outlined, atrophic, yellowish plaques on the legs was recognized by Urbach (1932) in a 44-year-old female patient with diabetes mellitus. In the biopsy specimen Urbach recognized what he believed to be necrobiosis in addition to extracellular accumulations of lipid. Hence the condition was given the name *necrobiosis lipoidica diabeticorum.* Lesions of the type described by Urbach had been reported previously by several authors beginning in 1927. In the subsequent twenty-five years the entity known as necrobiosis lipoidica diabeticorum has been found to occur not uncommonly in diabetics, and several clinical variants of the original description by Urbach have been recognized. Furthermore, there are many reports of necrobiosis lipoidica diabeticorum in nondiabetics, and there are examples of the disorder in diabetics in which no lipid can be demonstrated in the sections. The terminology used by Hare (1955) should perhaps be adopted: *necrobiosis lipoidica diabeticorum,* when diabetes mellitus is

present, and *necrobiosis lipoidica*, when the disorder occurs in nondiabetics.

ETIOLOGY AND PATHOLOGIC PHYSIOLOGY. The pathogenesis of necrobiosis lipoidica (diabeticorum) is not known and the early beliefs that the disorder was a local disturbance of lipid metabolism appear to be based on little or no evidence. Extracellular lipid accumulation is not consistently found in either the diabetic or nondiabetic types, and there is no elevation of any of the serum lipids.

The occurrence or progression of necrobiosis lipoidica diabeticorum does not appear to be associated with the severity of the diabetes and the lesions persist despite adequate control of the diabetes. Furthermore, the notion that this disorder has any prophetic significance for the development of diabetes is difficult to believe, in view of the fact that typical lesions proved histologically have been followed for 20 years or more without the occurrence of diabetes. Although there are no reliable statistical data available, there is an increasing belief among dermatologists that this disorder occurs with equal frequency in nondiabetics and diabetics.

Urbach, in the original case, recognized changes in the dermal arteriolar vessels, including thickening of the walls and intimal proliferation. Although these vessel changes have been emphasized in many of the subsequent reports, the vascular changes are not invariably present. Hare (1955) has emphasized the difficulty of explaining the necrobiotic areas as "infarcts" in view of the extensive collateral circulation of the dermis provided by extensive anastomosing subpapillary and dermal plexuses.

Finally, it must be stated that the pathogenesis of necrobiosis lipoidica (diabeticorum) has not been elucidated. The occurrence of glycogen in the lesions of both the diabetic and nondiabetic types remains an isolated and interesting finding (Hare, 1955). Glycogen occurred not only in the histiocytes, but in the tissue spaces surrounding the necrobiotic areas.

External trauma has been mentioned as an exacerbating factor in several reports. One of my cases was a cowboy who developed lesions on the inner aspects of the lower legs where there was constant shearing skin contact while on a horse.

SYMPTOMS, SIGNS, AND LABORATORY FINDINGS. Necrobiosis lipoidica (diabeticorum) is more common in females and in young adults, two thirds of the patients are below the age of 40 years. The lesions are ordinarily asymptomatic.

The large majority of the lesions are located on the leg, often at sites of trauma, and the patient may give a history of an insect bite, a bruise,

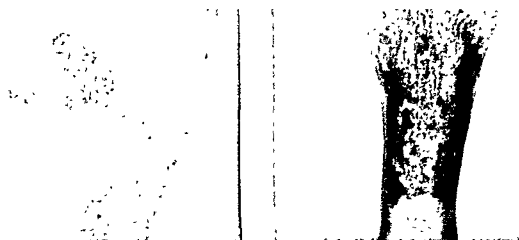




Plate 38-1

Plate 42-1. Necrobiosis lipoidica. (A) Characteristic shiny, atrophic plaque. (B) Extensive involvement of the leg with ulcerations and usually diagnosed as chronic venous insufficiency with dermatitis and ulcers.

Plate 38-1. Extravasated blood in deep layers of callus.

or a scratch. The lesions may be unilateral or bilateral and on first examining the patient one is often impressed by their delicate beauty (Plate 42-1A). They stand out in sharp contrast from the surrounding skin, have a polycyclic or arciform configuration, and appear as shiny, "varnished," atrophic, pink, faintly yellow plaques traversed by fine arborizing capillaries and surrounded by a definite red-brown or pink border. On diascopy (pressure with glass slide) a yellow color is usually detectable. The lesions start as small papules that gradually enlarge to rather large plaques, occasionally covering most of the surface area of the lower leg. Most plaques, however, are less than 10 cm. in diameter. The plaques are characteristically firm and platelike, very much like localized scleroderma. Ulcers are not uncommon sequelae and are indolent and usually painless (Plate 42-1B). A clinical variant of the above rather typical description is the nodular form (Fig. 42-4), which may simulate noduloulcerative tertiary syphilis, granuloma annulare, or sarcoidosis.

The clinical lesion is so typical in most instances that the disorder is easily recognized.

Although there is considerable variation in the histologic pattern of necrobiosis lipoidica (diabeticorum), certain basic features are usually present. The most striking change is the scattered areas of necrobiosis of collagenous connective tissue, which assumes a homogenous and degenerative state (fibrinoid) although its structural pattern remains. The elastic tissue is often well preserved in the areas of necrobiosis. Extracellular lipid deposits are often but not always present. The lipid is thought to be a phospholipid or cholesterol. The lipid may also be contained in foam cells but no true Touton giant cells, as seen in xanthoma, have been described. It is possible that the lipid may be responsible for the sarcoidlike inflammatory response (consisting of giant cells, lymphocytes, plasma cells, and histiocytes) occasionally seen. Obliterative changes in the small and medium-sized blood vessels are usually found, and some reports have emphasized the vascular changes as primary factors in the pathogenesis. However, as discussed previously, the absence of vascular changes in some patients and the well-developed collateral circulation of the dermal vascular tree suggest that the necrobiosis is not the result of anoxia or infarction based on vascular occlusion.

DIAGNOSIS. The clinical and histologic features are usually pathognomonic of necrobiosis lipoidica (diabeticorum). The histologic and occasional clinical similarity (especially in the nodular form of necrobiosis lipoidica) to granuloma annulare may offer a problem in diagnosis, particularly if the nodules occur on the arms. The tuberculoid re-

sponse present in some lesions may suggest sarcoidosis. When ulcers are present, the disorder is often erroneously regarded as chronic *venous insufficiency.*

TREATMENT. The early enthusiasm for the use of vitamin E in necrobiosis lipoidica diabeticorum has waned. Injection of soluble hydrocortisone directly into the lesions has been used successfully but the



FIG. 42-4. Necrobiosis lipoidica. Nodular variety, simulating sarcoid or lymphoma.

remissions are temporary. Surgical excision and grafting have been used successfully in one case (Cawley and Dingman, 1951). The lesions ordinarily persist despite control of the diabetes mellitus. In one recent report (Silver and Zeligman, 1957), however, the lesions disappeared and recurred parallel with the control of the diabetes.

COURSE AND PROGNOSIS. The disorder may persist for more than 20 years or may undergo fibrosis and involution.

INSULIN LIPODYSTROPHY (syn. Lipoatrophy)

The loss of subcutaneous fat at the site of insulin injections was first reported shortly after the introduction of insulin therapy of diabetes. In the subsequent three decades there have appeared numerous case reports of insulin lipodystrophy and a few studies have been made on its pathogenesis. Insulin lipodystrophy is a relatively frequent complication and the reported incidences vary from 1 to 54 per cent of diabetics.

PATHOGENESIS. In an attempt to elucidate the pathogenesis of insulin lipodystrophy, most of the obvious factors have been considered. There is an apparent sex and age predilection since the majority of the cases have occurred in children and women. The relative immunity of the adult male has suggested to some clinicians that androgens may inhibit development of insulin lipodystrophy. Nevertheless, insulin lipodystrophy has been reported in hypogonadal adult males. It has been stated that the disorder is more common in patients with a history of allergy, but this has not been confirmed in a careful study of 186 patients studied by Paley (1953). The possibility of infection as a factor in its production does not appear likely in view of the almost complete lack of inflammatory infiltrate in the areas of atrophy. The subcutaneous fat atrophy occasionally seen in narcotic addicts invariably shows foci of chronic inflammatory cells and suggests infection as a factor in this type of fat atrophy. The role of repeated trauma in the same site of injection has been proposed and an experimental study of fat atrophy following the injection of insulin into the fat pads of partially depancreatized male rats has been reported. However, control injections were not used in this study and in a subsequent repetition of this experiment, using alloxan diabetic animals and control injections with saline, no subcutaneous fat atrophy occurred at the site of the insulin injections. Various added or indigenous chemicals present in insulin preparations have been implicated in the production of the subcutaneous fat atrophy. The amount of lipase in commercial preparations of insulin is negligible and cannot be a factor in the production of fat atrophy. Since insulin lipodystrophy has been reported with all types of insulin, the low pH of soluble insulin (pH 2.5 to 3.0) cannot be assumed to play a role. In one series (Paley, 1953) there was a significantly higher incidence of lipodystrophy in patients receiving zinc protamine insulin. Finally, Paley (1953) has revived the idea that lipodystrophy may result from a local allergic phenomenon. He noted that 60 per cent of the diabetics with lipodystrophy were found to have "dermal reactions" to protamine zinc insulin while 27 per cent had dermal reactions to soluble insulin.

Statistical analysis using the X^2 test of his data indicated a significant association of dermal reaction and lipodystrophy ($p = 0.01$); however, the X^2 test does not measure the degree of association.

A curious improvement in lesions occurred in one series (Collens, *et al.*, 1949) despite continued injection of insulin into the lipoatrophic areas.

CLINICAL FEATURES. A localized area of loss of subcutaneous fat appears depressed at first and later the underlying muscle can be palpated. The depressions may be small (2 to 4 cm.) and only slightly depressed. There is no discoloration or induration. The atrophy may appear in areas away from the site of injection. Lipohypertrophy has also been reported and is more commonly seen in males.

COURSE AND TREATMENT. Insulin lipodystrophy is not important except from a cosmetic aspect. The atrophic areas disappear in many patients if the insulin injections are stopped and given in other areas. Most of the suggestions for prevention of insulin lipodystrophy have not been successful. For example, administration of insulin that has been stored at room temperature has not appeared to have changed the incidence. Deep intramuscular in lieu of subcutaneous injection is not associated with insulin lipodystrophy, but is not appealing to most diabetics. The most practical suggestions include the injection of insulin in areas that will not be subsequently exposed to the public and the adoption of a rotation of injections so that a given area does not receive insulin oftener than once a month.

PRURITUS

A persistent and generalized pruritus has been noted frequently enough in patients with diabetes mellitus so that the association perhaps can be considered more than coincidental. However, there is apparently no clear-cut relationship between the pruritus and the severity of the diabetes and the itching does not always disappear with adequate control of the metabolic disturbance. Injection of some of the metabolites found in the diabetic (acetone, glucose, acetoacetic acid) into the cisterna of rabbits and cats failed to elicit a scratch response (Koenigstein, 1948). It is possible that many of the examples of generalized pruritus reported in diabetics may have been due to other causes that were not excluded: uremia, liver disease, lymphoma, asteatosis ("dry skin"), drugs.

Localized pruritus, especially of the vulva, must be assumed to be diabetic in origin until proved otherwise, and may be the first symptom of diabetes. Severe intractable pruritus vulvae may occur in diabetics

even in the absence of candidiasis (see earlier discussion). The mechanism of the pruritus in the vulva in diabetics has not been elucidated. It cannot be explained by a local prurigenic response elicited by contact of the glucose in the urine since application of glucose to the vulva does not cause pruritus in the absence of candidiasis.

CAROTENEMIA

Carotenemia, usually associated with yellowing of the skin, is a condition in which the concentration of blood carotene is abnormally high. Most of the yellow component of normal skin is contributed by carotene, and its presence can be recognized by its characteristic absorption spectra in the blue region of the skin spectrum at 4820 A. Carotene is the prominent yellow coloring matter found in carrots, squash, pumpkins, sweet potatoes, beans, oranges, and palm oil. Normally, it is converted in the liver to vitamin A. Carotene is principally deposited in the stratum corneum and, therefore, the most intense coloring occurs in the palms and soles. The majority of diabetics have increased serum concentrations of carotene, although only 10 per cent show a detectable yellowing of the skin. The high incidence of carotenemia among diabetics is believed to be due to the high lipochrome diet and the impaired conversion of carotene to vitamin A. Carotenemia is also noted in association with myxedema, panhypopituitarism, male hypogonadism, and, occasionally, liver disease.

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Chapter 43

PROGNOSIS IN IDIOPATHIC DIABETES MELLITUS

Howard F. Root

GENERAL CONSIDERATIONS

Idiopathic diabetes mellitus may be defined as a hereditary disorder characterized by the presence of dextrose in the urine and persistent hyperglycemia. It is not equivalent to the hyperglycemia accompanying such conditions as pheochromocytoma, pancreatectomy for carcinoma, or hemochromatosis. However, the major clinical features of diabetes mellitus are dependent upon hypoinsulinism, resulting from deficient production, reduced effectiveness, or increased destruction of insulin secreted by the islands of Langerhans. The diabetic pattern, in the anthropologic sense, is based upon the hereditary transmission of pancreatic inferiority. Other genic factors appear to play a part in many cases, and particularly to affect the prognosis because of their influence, as yet not clearly defined, upon sequelae of diabetes in the nervous system, the endocrine system, and particularly in the vascular system. The influence of hereditary factors is most strikingly seen in the high incidence and the early development of changes in carbohydrate tolerance and actual development of clinical diabetes in children born to young diabetic mothers, especially if those mothers have had diabetes

a number of years before the occurrence of pregnancy. Indeed this fact, together with the now generally recognized fact that women who bear babies of excessive weight are destined to develop diabetes within 10 to 20 years thereafter, has raised the question as to whether diabetes is always inborn and present at birth even though its clinical manifestations may not appear for many years thereafter. Such conditions as chronic pancreatitis with calculus formation, or hemochromatosis, or the traumatic effects of hyperactivity of such glands as the pituitary or the adrenals, may be more effective in instances where the pancreas is already inferior by reason of hereditary influences.

It may be said that the prognosis in diabetes depends upon certain general factors as well as upon the specific character of diabetes itself. The general physical condition of patients, entirely apart from the diabetes, is a first consideration. Then the intelligence, disposition, and ability of the patient, whether young or old, to carry out orders, and the opportunity to execute the regime required by the physician are of major importance. The possibility of control depends upon the initial severity as indicated by appropriate studies; and a final, most important consideration is the character of medical treatment and supervision over the many years of its course that the physician may provide.

The presence of obesity and a favorable hereditary background, together with early diagnosis and prompt treatment, are valuable and favorable prognostic signs. Patients who appear in critical condition at the first visit with acidosis owing to some infection or complication, or with high blood values, may prove most favorable and may even have remission. It is unwise to give a positive statement about prognosis until after an opportunity to carry out treatment and to observe the patient under proper conditions has been attained. Today, diabetic patients suffer from, and finally die chiefly of, complicating disorders or the sequelae of inadequate treatment rather than of diabetes itself.

TYPES OF DIABETES

The classification of diabetic patients according to various types has never been entirely satisfactory. In general, all students have agreed that diabetes beginning in childhood is relatively severe; whereas diabetes of the obese, middle-aged patient may remain mild for many years. Nevertheless, the once severe case often with time becomes so mild that symptoms disappear and insulin may be omitted for years at a time. It is certainly true that in the majority of patients in whom the disease begins after the age of 30 years, diabetes is essentially a mild disorder that may remain mild in some patients, with or without treatment. This

group of patients with mild disease and, frequently with obesity, endanger themselves and many other patients by their apparent success in neglecting doctors' advice until 20 years later, when *retinitis* or gangrene occurs. In some instances, however, this mild diabetic stage may go on without treatment until death occurs from an auto accident or some unrelated disease.

The fact remains, however, that although no necessity compels the progression from mild to moderate or severe diabetes, it is from this supposedly mild group of middle-aged patients who receive no insulin or careful dietary treatment that a considerable percentage of the severe foot complications later develop.

The question of classification is involved in the basic concept of diabetes as a unitary disorder with heredity as the one common factor.

Severity of diabetes may be measured in a variety of ways. The units of insulin required may be employed as a temporary measure of severity. Patients who can be sugar-free on an adequate diet without insulin have temporarily less severe diabetes than patients requiring a large amount of insulin. Differences of insulin doses are notably unreliable since many temporary factors will alter the dose. Thus infection, variations in diet, or temporary acidosis may require a large dose, which a few months later is greatly reduced. Patients who are sensitive to insulin may be contrasted with those who are relatively resistant. Again, insulin resistance may be temporary.

Acidosis or coma may develop in the mildest case of diabetes and is in no sense a measure of the basic severity of the diabetes. Nevertheless, the young labile patient who develops ketosis easily is generally to be regarded as having fundamentally severe diabetes.

Insulin production by the pancreas may prove, when new methods make its determination more easy, a fundamental measure of severity. Certainly children commonly become complete diabetics after three years of the disease and then little or no insulin can be recovered from the pancreas or the blood. In contrast, middle-aged, mild, and obese diabetics have been found to possess 50 to 70 per cent of the normal content of insulin in blood and pancreas (2, 16) (Chaps. 31 and 32).

A mild diabetic may be said to be one whose urine is sugar-free before and after meals with a diet containing carbohydrate 150 gm. in 24 hours without taking any insulin. It is almost unfortunate to use the word "mild" since it encourages indifference in the patient. Vascular complications in this group are frequent.

A moderate diabetic might be considered one whose urine is sugar-free with 100 gm. carbohydrate in 24 hours with 10 to 20 units daily. A severe diabetic might be described as a patient who could not be

sugar-free without insulin and would require 50 or more units daily to maintain a sugar-free urine with a daily diet providing 150 gm. carbohydrate.

At present the severity of diabetes may best be measured by the tendency of the patient to develop the specific diabetic sequelae in the vascular system, the retina, and the kidneys. It follows that, in general, the severity of diabetes is more closely related to the age of onset of clinical diabetes than to any other single measure. Thus proliferating retinopathy occurs with greatest frequency in the diabetes that begins in childhood or before the thirtieth year. It is, sooner or later, associated with the diabetic nephropathy and premature occlusive arterial disease characteristic as specific effects of insulin deficiency and the other metabolic deficiencies that together comprise the disease diabetes mellitus. If one accepts the age at onset as of chief importance, then the prognosis in terms of years of life is best in youth but less favorable in terms of the typical diabetic complications.

Early diagnosis is of prime importance at any age. It is still true that 1 in 7 patients with diabetic coma have no diagnosis of diabetes until grave acidosis and coma have developed. In middle life, patients frequently come for medical examination because of loss of vision or severe foot lesions whose diagnosis of diabetes could have been made and to whom appropriate treatment might have been given 5 or 10 years earlier. Delay in diagnosis still poses a problem that can only be met when the diabetes detection program of the American Diabetes Association and the combined efforts of all physicians succeed in persuading everyone to have a urine test and a health examination at least once a year.

DURATION OF LIFE AND PROGNOSIS

The relation of prognosis to improvement in the quality of treatment is the basis for the general belief that early adequate treatment will prolong life and postpone complications. In Table 43-1, a contrast is presented between the duration of life after onset of diabetes in the years preceding the introduction of insulin in 1922 and the duration of life of deceased patients during the period from 1950 to 1957 (6). An increase in the duration of life from an average from 4.9 years in the Naunyn Era to 6.1 in the Allen Era resulted from improvement in control of diabetes wrought by the Allen Treatment by undernutrition. During the years that followed the introduction and use of insulin the average duration of life for patients for whom the disease was fatal steadily increased as the use of insulin became more widespread and

more skillful. Other factors were a better general understanding of diabetic dietary treatment and advances in the treatment of infections and surgical complications. During the Charles H. Best Era, here divided into 2 periods, the best results as yet attained are shown: in the period ending December 31, 1955, the average duration of life for 4,376 patients was 15.6 years and in the two-year period ending December 11, 1957, the average had *risen to 18.2.* The general outlook for duration of life is, therefore, still steadily increasing. The figures are

TABLE 43-1. DURATION OF LIFE SUBSEQUENT TO ONSET OF DIABETES AMONG DECEASED DIABETIC PATIENTS IN IMPORTANT ERAS OF TREATMENT*
Number and Percentage of Cases Classified According to Duration

	Naunyn Era (1897 to 5/31/14)		*Allen Era (6/1/14-to 8/6/22)*		*Charles H. Best Era (1/1/50 to 12/31/55)*		*(1/1/56 to 12/11/57)*	
Age groups at onset	*Number of cases*	*Duration years*	*Number of cases*	*Duration years*	*Number of cases*	*Duration years*	*Number of cases*	*Duration years*
All ages	326	4.9*	836	6.1*	4,376	15.6+	640	18.2+
0-9	24	1.3	61	2.9	107	20.6	30	26.4
10-19	39	2.7	81	2.7	198	20.1	52	23.5
20-39	85	4.3	215	4.9	621	21.8	91	25.1
40-59	126	7.0	351	8.0	2,284	16.2	304	18.7
60 and over	51	4.1	117	6.4	1,161	10.0	159	10.2
Unknown	1	—	8	—	5	—	4	—

* Experience of the Joslin Clinic, 1897–1957.

even more impressive when attention is given to the groups with onset of diabetes early in life. The duration of life of children has increased tenfold. One may say that the expectation of life for diabetic patients has increased at a rate much greater than that of the general population.

For some years it has been a practice at the Joslin Clinic to award a medal to any patient who lives longer after the onset of diabetes than he would be expected to live without it according to life-insurance tables. Already nearly 3,000 such patients have been found among 44,000 true diabetic patients.

It is true that diabetic death rates, at all ages, are greater than for the general population. However, the diabetic deaths recorded in the census of the registration area of continental United States do not in-

clude the total number of diabetics who die. Failure to include diabetes on the death certificate occurs in one quarter of all diabetics (8). The error on the side of understatement is significant. For males the diabetic rate is fully three times as great up to the age of 50, and, thereafter, twice as great. For females, the diabetic mortality rate is 4 to 5 times as large as the normal. Nevertheless, the average diabetic in recent years has increased his expectation of life greatly, and in the future can expect to survive at least three fourths as long as the average individual. Even this figure may be an underestimation as one reviews the records of the many living patients who are now active after 35 or more years of diabetes. A series of 245 patients who had survived more than 35 years of diabetes to January, 1957, has grown to exceed 300 by July 1, 1958 (12, 13).

Juvenile diabetics, with onset of diabetes from infancy to the fifteenth birthday, numbered 3,732, up to August 1, 1955. Of these, 1072 patients had survived 20 years of diabetes since onset, and 879 were living (15). Among these living patients, 24 per cent had had diabetes between 30 and 34 years, and 40 per cent had had diabetes from 25 to 29 years.

Diabetes with onset of the disease under 40 years of age represents about one third of all cases (7). Among this group were 760 patients with diabetes of 25 years or more duration whom Joslin divided into 4 groups (7):

1. Quarter-Century Victory-Medal diabetics, numbering 23;
2. Those with onset in childhood who have survived more than 30 years, numbering 40,
3. Those with onset in childhood who have had disease between 25 and 30 years, numbering 181;
4. Those whose onset was between the ages of 15 and 40 and who have survived 25 years or more, numbering 516.

The survival of these Quarter-Century patients was not a matter of coincidence. The more closely their histories were studied, the more definite were the explanations for their long careers. Many were fortunate in their economic and social status; a few were from physicians' families. In general, continuity of medical care under a single physician was an important feature. Males and females were about equal in number. The incidence of diabetes in other members of the family averaged 44 per cent for the group.

The winners of the Victory Medal were conspicuous for the strenuous control of their diabetes in early years. Complications of the diabetic type had occurred, but only after long duration of diabetes.

QUALITY OF TREATMENT

All experience agrees that the delay in the diagnosis of diabetes carries special dangers: first, in youthful patients; and second, in patients with onset of diabetes late in life. Untreated diabetes *in early life* has always, and still does, carry the risk of the rapid development of diabetic coma and death. Even if acidosis is not fatal it may leave in a considerable percentage of patients a moderately severe diabetes, with a correspondingly less favorable prognosis than would have been the case if *vigorous treatment with insulin and diet had been initiated* days or weeks earlier. In middle and later life, delay in diagnosis encourages the premature development of vascular complications in heart, legs, and eyes. Once the diagnosis is made, then it is the quality of treatment and the degree of control of the diabetic state that is of *basic importance in determining both length of life and development of* serious diabetic sequelae. At present, we cannot define "control of diabetes" in accurate biologic or biochemical terms; but clinical control is a generally accepted objective. In a strict sense, the control of diabetes should produce a complete reversion of the condition and maintenance of chemical and metabolic elements in a strictly physiologic balance. Control would, therefore, mean the maintenance of the normal status such as existed before diabetes developed. Although such perfect control for a lifetime is a human impossibility at the present time, definite data clearly show that persistent efforts to maintain such control will go far toward preventing diabetic complications and toward prolonging life. In recent years, at the Joslin Clinic, a study with respect to the influence of control upon the course of diabetes has been carried out in 451 patients with diabetes beginning in childhood or before the age of 25 years (9, 14). Each patient was studied by roentgen ray for the presence or absence of calcified arteries, and had an ophthalmologic examination; in addition, a complete physical examination and tests of urine and blood were made. The patients were classified according to the following criteria of diabetic control:

CRITERIA OF DIABETIC CONTROL

Excellent Control

1. The patient must never have been in coma.
2. Insulin administration must have been started within a few weeks of onset of diabetes.
3. Urine tests for sugar must have been made *more than once daily ever since* onset of diabetes, with a conscientious attempt to have the urine sugar-free or nearly so before meals, and with insulin dosage adjustments determined by *the results of the urine tests.*

4. The diet must have been weighed at least 60 per cent of the time since the onset of symptoms of diabetes.
5 Regular physical examination and laboratory tests by a physician must have been faithfully completed at least once annually, and blood and urine tests must have been satisfactory.

Good Control

1. The patient must never have been in coma, except in instances in which the initial diagnosis of diabetes was made when the patient was in coma.
2. Insulin administration must have been started within a few weeks after onset of diabetes.
3. Urine tests for sugar must have been made at least once daily ever since onset of diabetes, with a conscientious attempt to have the urine sugar free or nearly so before meals, and with insulin dosage adjustments made according to results of urine tests.
4. Diet must have been weighed for the first six weeks of treatment and thereafter at intervals, with careful measurement of food at all other times since onset of diabetes.
5. Regular physical examination and laboratory tests by a physician must have been done at least once annually, and blood and urine tests must have been satisfactory.

Fair Control

1 The patient must never have been in coma, except for cases in which the diagnosis was made with patient in coma, or in rare cases in which an unavoidable overwhelming infection or complication precipitated coma.
2. Insulin administration must have been started within 24 months of onset of diabetes.
3 Tests of the urine for sugar must have been made one or more times weekly in an attempt to have sugar-free urine tests.
4. Dietary management by the patient must have been conscientiously attempted, although food was not weighed or measured, and the patient must have rarely, if ever, indulged in gross dietary indiscretions.
5. Satisfactory blood sugar determinations must have been made at the time of physical examinations by the patient's physician at least once every two years.

Poor Control

1. Avoidable coma that occurred one or more times.
2 Insulin administration was not started until more than 24 months after onset of diabetes, and, in some cases, insulin was taken irregularly.
3 Urine specimens were tested infrequently or at intervals of months or years.
4 No measurement or weighing of food in relation to urine tests or insulin dosage was done
5. The patient had no regular examinations by a physician, and office or hospital examinations done at long intervals showed severe glycosuria and hyperglycemia

The results of such classification are shown in two tables. In Table 43-2, it is seen that in 11 patients under excellent control throughout the entire period, no nephropathy occurred; in 50 patients under good control, only 1 patient was found with albuminuria. The incidence of nephropathy increased to 17 per cent among 90 patients with fair

control, and to 28 per cent among 298 patients whose control had been poor.

Equally striking was the effect of control upon the incidence of retinitis. In 189 patients with diabetes of 20 to 29 years' duration, the relation of control of diabetes to the incidence and severity of diabetes appears in Table 43-3.

TABLE 43-2. NEPHROPATHY IN 451 PATIENTS WITH DIABETES OF TEN TO THIRTY-FOUR YEARS' DURATION

Degree of control	*Number of cases*	*Nephropathy, percentage of cases*
Excellent	11	0
Good	50	2
Fair	90	17
Poor (medical advice neglected)	298	28

TABLE 43-3. RETINITIS IN 189 PATIENTS WITH DIABETES OF TWENTY TO TWENTY-NINE YEARS' DURATION

		Percentage of cases, each degree of retinitis				
Degree of control	*Number of cases*	*None*	*Slight*	*Moderate*	*Marked*	*Extreme*
Good	32	45	31	20	4	0
Fair	41	34	18	37	2	9
Poor	116	16	17	31	19	17

Thus in 32 patients with good control over this long period of time no retinal hemorrhages or exudates were present in 45 per cent of the cases. On the other hand, among 116 patients with poor control, even though taking insulin, only 16 per cent were free from retinitis. Among the 32 cases of good control, none had extreme retinitis, while 17 per cent of patients with poor control showed far advanced retinitis proliferans. Equally convincing are the studies of Jackson (4, 11) and his co-workers who have analyzed the course of juvenile diabetic patients studied at the University of Iowa. A careful comparison of the relative importance of the long duration of diabetes, as compared with the influence with the degree of control, was made employing accurate statistical methods. They concluded that the incidence of diabetic sequelae, namely, retinitis, nephropathy and arterial sclerosis, was more definitely related to the control of diabetes than to the duration of the disease.

It must be emphasized that the word "control" means much more than a sugar-free urine test; it includes early diagnosis and the use of insulin; continuous, adequate, medical supervision and utilization of the best methods of general medicine; the prescription and accurate measurement of an adequate diet; finally, the daily attempt to prevent excessive hyperglycemia and glycosuria.

CAUSES OF DEATH

As evidence of continuing improvement in treatment and consequent hope for the future, the increasing duration of life is second only to the extraordinary changes in causes of death in diabetic patients during the present century (6). In Table 43-4, the causes of death of 18,055 diabetic patients are arranged according to three periods: the first, prior to the use of insulin in 1922; the second, from 1922 to 1950; and the

TABLE 43-4. CAUSES OF DEATH OF 18,055 DIABETIC PATIENTS*†

	Naunyn-Allen era 1898–Aug. 1922		*Banting-Hagedorn era 1922–12/31/1949*		*Charles H. Best era 1950–12/11/1957*	
Cause of death	*Deaths*	*Percentage of all cases*	*Deaths*	*Percentage of all cases*	*Deaths*	*Percentage of all cases*
All Causes	1162	100 0	11,877	100 0	5016	100 0
A Coma present	555	47.7	536	4 5	62	1.2
B. Coma absent						
1. Cardiorenal-vascular	263	22 6	7569	63.6	3837	76.5
Arteriosclerotic	260	21 1	7509	62 8	3805	75.9
Other circulatory and rheumatic heart disease	3	1.5	60	0 8	32	0.6
2 Infections, total	130	11 2	1181	10.0	260	5 2
3 Cancer	37	3 2	1088	9 2	524	10 4
4 Tuberculosis	57	4 9	318	2.7	33	0.7
5 Diabetes (i e , unknown)	64	5 5	367	3.1	31	0 7
6 Accidents	7	0 6	238	2.0	98	1.9
7. Inanition	19	1 7	6	0 1	2	0
8. Suicides	3	0 3	75	0 5	24	0.5
9 Insulin reactions	0	0	25	0.2	10	0 2
10. Other disease	27	2 3	501	4.1	135	2.7

* E Joslin, H F. Root, P White, and A. M. Marble, *Treatment of Diabetes Mellitus*, 10th ed. Philadelphia, Lea & Febiger, 1959, p. 7.

† Experience of Joslin Clinic, Boston, Massachusetts

third, from 1950 to December 11, 1957. Diabetic coma caused 63.8 per cent of 326 deaths prior to 1914, but for the Naunyn-Allen Era ending in August, 1922, coma was the cause of death in 47.7 per cent. Among 5,016 deaths in the Charles H. Best Era, coma was present in only 1.2 per cent. In our experience, a diabetic's chance for recovery from coma, uncomplicated, is 99 per cent; and his chance of dying with coma is today less than 1 per cent. Nevertheless, the danger of diabetic ketosis is still ever-present, and its influence upon the outcome of infections, serious surgery, and upon the development of diabetic complications is still a matter of major importance.

The prevention of diabetic ketosis and coma by early detection and treatment of diabetes by better methods of public information, by better teaching and training of patients in the control of diabetes and the prevention of ketosis has, therefore, a position of prime importance in improving prognosis for future diabetic patients.

The frequency of arteriosclerotic cardiorenal-vascular deaths has strikingly increased from 22.6 per cent in the earlier period to 75.9 in recent years. This change bespeaks the longer duration of life, but also the aging of the general population and more frequent diagnosis of diabetes. Unfortunately, errors exist that belittle the figure, because many patients with known diabetes are certified at death for cardiac or other causes without mentioning diabetes. Much can be done in the future to prevent and postpone many of these deaths which today occur too early, owing in part to inadequate diabetic control.

The decline in infections as a cause of death amounts to more than 50 per cent, and tuberculosis as a cause of death among our diabetics has almost disappeared. Cancer, on the other hand, has trebled, from 3.2 in the early period to 10.4 per cent in the recent period, again owing partly to the increasing age of patients and partly to better diagnostic methods. Diabetes alone is gradually disappearing as a diagnosis. Accidents, inanition, suicides are few. Recent studies indicate that an individual who secures careful training in the management of the disease may be a safer person as an auto driver than his nondiabetic brother. Insulin reactions, usually the result of unfortunate error, should disappear as the cause of death.

REMISSIONS

Although the tendency to diabetes is hereditary, inborn, and permanent, remarkable variations in its severity and manifestations occur. In some, it will appear only as glycosuria and hyperglycemia under temporary stresses, such as pregnancy or infections, at long intervals, or

through overactivity of other endocrine glands. However, it is not usually intended to include such periods of freedom from glycosuria as remissions, but to use this term in patients who have had outspoken clinical diabetes, and then undergo a remission. A number of children have been observed with acute diabetic symptoms, including hyperglycemia and acidosis, who following a few days or weeks of treatment with insulin and diet may become normal-glycemic and without insulin may continue for months or years on a diet only moderately restricted. In the past, either intercurrent infections or the patient's overindulgence in food has brought back manifestations of diabetes. Such remissions are somewhat more frequent in adults and may persist for much longer periods. In addition, one may speak of remissions in which a patient requiring 50 or more units of insulin may rather abruptly cease to require more than a fraction of his previous dose. Again, such remissions probably would last for long periods if carefully measured dietary treatment was continued. Among the causes of remissions may be mentioned certain types of liver disease and endocrine disorders such as hypoadrenalism. Even after years of treatment, and particularly with the more skillful use of insulin and diet, the possibility of such remissions may greatly improve the prognosis of many diabetic patients.

CURES

Criteria for cure must rest upon standards of diagnosis and tests for recovery. As yet, we have not encountered a cure, but prefer to retain the use of the word "remission." However, the following standards may be proposed.

1. Diagnosis. The diagnosis shall rest upon glycosuria of 0.5 per cent or more accompanied by a fasting blood sugar of at least 110 mg. per cent (true glucose) of a venous blood sugar after a meal or on a glucose tolerance test of at least 1.50 mg. per cent (true glucose).

2. Duration of proved diabetes. Duration of diabetes shall be recorded in months by repetition of the above tests.

3. Test for recovery. Glycosuria and hyperglycemia should be absent while the patient is without diabetic medication both before and one hour after a meal containing 75 to 100 gm. carbohydrate. The carbohydrate tolerance test should be normal following the oral administration of 100 gm. glucose to the patient in a fasting state. A proved case of diabetes that conforms to the test of recovery at the beginning and end of an interval of 5 or more years may be considered cured.

Although measures for prevention of diabetes and of its sequelae are regarded most hopefully, cases of cured diabetes occasionally reported

in recent years are generally viewed as not being instances of true idiopathic diabetes. The word "cure" is not found in the index of Danowski's textbook (3). Kiss and Barta (10) refer to a summary by Arey (1) of cured cases from 7 authors. Yet they cautiously consider that exogenous factors, including infections or toxic effects upon some cerebral center, may have been the cause of such temporary diabetes-like states, rather than true diabetes mellitus.

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Chapter 44

PREGNANCY IN THE DIABETIC

Joseph H. Crampton

Prior to the discovery of insulin, few women who had c betes in childhood lived to reach the childbearing age, and among th who did survive, fertility was lowered. Successful pregnancy was alm entirely confined to the mildly diabetic adult woman becoming pregn later in life, and to the relatively unusual diabetic who noted the or of her disease during pregnancy. With the discovery of insulin and increased refinement in management of the juvenile diabetic during last three decades, a larger number of girls with diabetes of m years' duration are reaching the childbearing age. Advances in ther have also resulted in normal fertility. The net result is a greater quency of pregnancy in diabetic women, particularly in those who h had diabetes for a number of years and who are therefore prone exhibit accompanying vascular disease. Vascular damage is, in t associated with an increase in complications which may lead to de of the fetus. Thus, advances in the treatment of diabetes have multip the problems for the physician faced with the care of the pregr diabetic.

ALTERED PHYSIOLOGY DURING PREGNANCY

PREDIABETES AND PREGNANCY. Diabetic women exhibit an obstetı history many years prior to the onset of their disease that is simila

that after the onset of frank diabetes. The prediabetic woman exhibits a remarkable propensity to have large babies, or stillborn fetuses, or children dying in early infancy; during pregnancy she is prone to exhibit toxemia and excessive hydramnios. The occurrence of large infants has been cited as evidence of excessive growth hormone production. During the 5-year period immediately prior to onset of diabetes, the incidence of fetal loss is comparable to that following the onset of the disease. Demonstrable as early as 20 years prior to onset, excessive fetal loss increases progressively as the time of appearance of clinical diabetes is approached. The high incidence of fetal loss in the prediabetic woman strongly indicates that control of the diabetes alone will not guarantee the diabetic a living child.

Women with a history of excessively large babies or of late pregnancy accidents or those exhibiting diabetic glucose tolerance tests during pregnancy should be treated in the same manner as those with manifest diabetes.

Prediabetes in the father, according to Jackson, is associated with an increased incidence of overweight babies. Babbott, on the other hand, found no increase in the size of the offspring of diabetic fathers. The wives of these men did have a somewhat increased incidence of spontaneous abortions.

PREGNANCY AND THE INCIDENCE OF DIABETES. Diabetes is more common in women who have been previously pregnant. Statistical studies of this problem have suggested that the increased incidence of diabetes in women is largely associated with previous pregnancy and that the incidence of diabetes is directly proportional to parity. The incidence of diabetes in postmenopausal women who have not borne children is very close to that of men in the same age group. It may be that pregnancy is a significant etiological factor in the incidence of diabetes in women.

EFFECT OF DIABETES ON FERTILITY. Diabetes is encountered about once in 500 pregnancies. This figure approximates the incidence of diabetes in women of [illegible] aring age. [illegible] 'c women whose diabetes is reasonably well [illegible] are no [illegible] ile.

mented by the lowered renal threshold for glucose, loss of carbohydrate in the urine may reach considerable proportions in the pregnant diabetic. This, in turn, leads to an osmotic diuresis not only of fluid but of accompanying electrolytes. The diabetic is thus put in a vulnerable position where even a slight upset, particularly if attended by nausea and vomiting, may precipitate acidosis, since carbohydrate intake is inhibited and loss of electrolytes is further increased.

The insulin requirement tends to rise during pregnancy in most patients. The over-all increase is often minimal and occasionally a decrease may occur. There is no evidence for the concept that the fetus tends to supply insulin for the mother during the last trimester. In fact, Davies and Lacey have shown that insulin does not pass the placental barrier. White has observed a fall in the insulin requirement in some patients after several pregnancies. The cause of this reduced need for insulin is not known.

TOXEMIA. The pregnant diabetic shows a propensity for fluid retention, toxemia, and hydramnios. These phenomena are certainly seen more frequently in those patients with demonstrable degrees of renal damage and vascular disease, suggesting that impaired renal function is an appreciable factor. The role of increased production of adrenal steroids, including aldosterone, in the etiology of toxemia in the pregnant diabetic is not yet delineated. In spite of measures taken for its prevention, toxemia has been noted in about 25 per cent of the diabetic pregnancies reported in this country (Bachman).

HORMONAL BALANCE. Aberrations from the normal hormonal relationships during pregnancy have been demonstrated, particularly by Smith and Smith and by White. These changes are similar to those seen in the toxemia of nondiabetics and include abnormally high serum and urine chorionic gonadotrophin concentrations and low urine estrogen and pregnanediol levels. The low estrogen and progesterone excretion have been explained on the basis of placental and syncytial cell degeneration. More difficult to explain is the increased chorionic gonadotrophin activity.

Reports of the histologic changes in the placenta are conflicting. It is possible that the normal aging process of the placenta may be accentuated in the diabetic.

VASCULAR DISEASE

A considerable proportion of diabetic women in the childbearing age are found to have been juvenile diabetics who have had diabetes for a number of years and who exhibit vascular disease of varying degrees.

that after the onset of frank diabetes. The prediabetic woman exhibits a remarkable propensity to have large babies, or stillborn fetuses, or children dying in early infancy; during pregnancy she is prone to exhibit toxemia and excessive hydramnios. The occurrence of large infants has been cited as evidence of excessive growth hormone production. During the 5-year period immediately prior to onset of diabetes, the incidence of fetal loss is comparable to that following the onset of the disease. Demonstrable as early as 20 years prior to onset, excessive fetal loss increases progressively as the time of appearance of clinical diabetes is approached. The high incidence of fetal loss in the prediabetic *woman strongly indicates that control of the diabetes alone will not* guarantee the diabetic a living child.

Women with a history of excessively large babies or of late pregnancy accidents or those exhibiting diabetic glucose tolerance tests during pregnancy should be treated in the same manner as those with manifest diabetes.

Prediabetes in the father, according to Jackson, is associated with an increased incidence of overweight babies. Babbott, on the other hand, found no increase in the size of the offspring of diabetic fathers. The wives of these men did have a somewhat increased incidence of spontaneous abortions.

PREGNANCY AND THE INCIDENCE OF DIABETES. Diabetes is more common in women who have been previously pregnant. Statistical studies of this problem have suggested that the increased incidence of diabetes in women is largely associated with previous pregnancy and that the incidence of diabetes is directly proportional to parity. The incidence of diabetes in postmenopausal women who have not borne children is very close to that of men in the same age group. It may be that pregnancy is a significant etiological factor in the incidence of diabetes in women.

EFFECT OF DIABETES ON FERTILITY. Diabetes is encountered about once in 500 pregnancies. This figure approximates the incidence of diabetes in women of childbearing age. Diabetic women whose diabetes is reasonably well controlled are normally fertile.

EFFECT OF PREGNANCY ON CARBOHYDRATE METABOLISM. Normal women show no decrease in glucose tolerance during pregnancy. The prediabetic exhibits lessened glucose tolerance during pregnancy, which reverts to normal after delivery. The aberration in glucose response in the prediabetic is apt to be more pronounced in each subsequent pregnancy. Eventually permanent diabetes may appear. This phenomenon has been compared to steroid diabetes and may be related to the increased level of glucocorticoids during normal pregnancy.

Renal glycosuria is a common finding in normal pregnancy. Aug-

mented by the lowered renal threshold for glucose, loss of carbohydrate in the urine may reach considerable proportions in the pregnant diabetic. This, in turn, leads to an osmotic diuresis not only of fluid but of accompanying electrolytes. The diabetic is thus put in a vulnerable position where even a slight upset, particularly if attended by nausea and vomiting, may precipitate acidosis, since carbohydrate intake is inhibited and loss of electrolytes is further increased.

The insulin requirement tends to rise during pregnancy in most patients. The over-all increase is often minimal and occasionally a decrease may occur. There is no evidence for the concept that the fetus tends to supply insulin for the mother during the last trimester. In fact, Davies and Lacey have shown that insulin does not pass the placental barrier. White has observed a fall in the insulin requirement in some patients after several pregnancies. The cause of this reduced need for insulin is not known.

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VASCULAR DISEASE

A considerable proportion of diabetic women in the childbearing age are found to have been juvenile diabetics who have had diabetes for a number of years and who exhibit vascular disease of varying degrees.

The degree of vascular damage is a significant problem in the diabetic woman's pregnancy. Diabetes of long standing and an increasing degree of vascular damage are associated with greater fetal loss. White has classified all of her pregnant diabetics according to the duration of the disease, the presence or absence of vascular damage and the degree thereof. This classification is helpful in comparing results from heterogeneous series (see Table 44-1).

TABLE 44-1. CLASSIFICATION OF DIABETIC MOTHER
(According to White)

CLASS A	*CLASS B*	*CLASS C*
By glucose tolerance only	Diabetes onset after 20 Duration less than 10 years No vascular lesions	Onset 10 to 19 years Duration 10 to 19 years No vascular lesions
CLASS D	***CLASS E***	***CLASS F***
Diabetes onset before 10 years of age Diabetes over 20 years Retinitis or calcified vessels in legs	Calcified pelvic arteries	Nephropathy

Retinopathy and nephropathy may progress during pregnancy, though occasionally an unexplained improvement may be noted.

MANAGEMENT OF THE DIABETIC DURING PREGNANCY

The aspects of pregnancy in the diabetic deserving most careful attention are: (1) Control of diabetes and prevention of acidosis; (2) prevention of toxemia and control of excessive hydramnios; (3) selection of the proper time for and mode of delivery.

Control of Diabetes and Prevention of Acidosis

DIET. As is true for diabetes under all circumstances, control of the disease during pregnancy is basically [illegible] between diet, insulin, and exercise. An accurate diet is [illegible] prefer the weighed diet since all oth[illegible] relatively [illegible]) The diet can be calculated on t[illegible]0 calories [illegible] body weight with the addition of [illegible]ent for [illegible] growth [illegible] the fetus.

This amount, of course, must be reduced if the patient is obese at the time of conception. Since the patient should gain a minimum of weight during the pregnancy (less than 20 pounds), the increase in her diet should not be excessive. At the same time there may be an appreciable loss of carbohydrate in the urine, which must be considered in arriving at a suitable diet. A minimum of 100 gm. of protein is advised. The carbohydrate is set at 185 to 200 gm. daily and fat is used to make up the remaining calories. Fat may be drastically reduced in the presence of obesity (see Chapter 33 on the calculation of the diet).

INSULIN. The insulin requirement throughout pregnancy is determined by fortnightly blood sugar determinations. Blood sugar tests may be required more frequently as the time of delivery approaches. The patient is instructed to test the urine daily for sugar so that both she and her physician can maintain some concept of the degree of glycosuria. Usually, during the first trimester, there is no increase in insulin requirement. Most patients at this time can be controlled on a basic mixture of intermediate insulin such at NPH or lente and a small dose of quick-acting insulin, either crystalline or semilente, in a proportion of about 4 to 1 given before breakfast. Generally, as pregnancy advances, the doses of both types of insulin must be increased. Occasionally adequate control cannot be attained by a single morning dose, and supplemental injections before lunch and supper may be necessary.

Because of the reduced renal threshold, the diabetic woman must be forewarned that she will no longer find her urine tests to be reliable guides to insulin dosage. If she tries to keep her urine sugar-free, she will suffer frequent severe insulin reactions leading her to abandon all attempts at rigid control.

After delivery there is a precipitous fall in the insulin requirement for 24 to 48 hours, followed by a gradual rise over a period of about 7 days to levels similar to those before pregnancy.

HYPEREMESIS. Nausea and vomiting, especially during the first trimester, increase the hazard of acidosis during pregnancy. Diabetic acidosis, when it is allowed to occur, is invariably associated with an increased fetal loss. Hospitalization with careful observation is necessary if hyperemesis becomes a real problem.

INFECTION. Because of its hazard in precipitating acidosis, infection must be scrupulously watched for and treated. Perhaps urinary tract infection is the greatest offender. Diabetic females, in general, are prone to urinary tract infection and there is a generally high incidence during pregnancy. It is suggested that the acute phase of treatment, guided by sensitivity studies, be followed by a period of prophylactic administration of sulfa compounds in moderate doses if toxemia or reduced renal

function is not present. The patient must be carefully cautioned to consult the physician during any intercurrent illness.

Prevention of Toxemia and Control of Hydramnios

At the initial prenatal visit, the patient should be placed on a diet rigidly restricted in sodium for the remainder of the pregnancy. This diet includes abstinence from all added sodium, avoidance of all foods with salt added in preparation (such as ham, bacon, pickles, and many canned foods), and the use of salt-free butter and bread. Our experience has firmly convinced us that rigid restriction of salt from the onset decreases the incidence of toxemia, lessens the tendency to excessive hydramnios and, in turn, enhances fetal survival. Awaiting the appearance of edema or early signs of toxemia is inadequate. If signs of toxemia appear despite the rigid salt restriction, particularly if there is a rapid gain in weight, diuretics should be administered. Previously, ammonium chloride, 2 gm. three times daily, was used; occasionally mercurial diuretics were given. A limited experience with chlorothiazide has indicated that this compound has a definite place in the management of the pregnant diabetic. It should be given in doses of 1 to 2 gm. daily in the presence of rapid weight gain, edema, excessive hydramnios, or increasing hypertension.

Selection of the Proper Time for and Mode of Delivery

Selection of the proper time for and mode of delivery are the factors that may most affect fetal survival rates and require the most knowledge and experience. In the more complicated diabetic pregnancies, allowing the pregnancy to go to full term will be accompanied by an increase in large, edematous babies who are often stillborn. On the other hand,
too early delivery will be attende [illegible] igh incidence of excessive
prematurity and neonatal death. P. [illegible] n the presence of [illegible]
nios, determination of the state o [illegible] n of the fetus [illegible]
problem. In our experience, ancilla [illegible] uch as x-rays t [illegible]
the presence of c [illegible] moral [illegible] ve not been [illegible] y
helpful. Clinical [illegible] ical ju [illegible] offer the [illegible] f
accurate determi [illegible] l age
In general, th [illegible] betic, [illegible] who has [illegible] es
for over 10 yea [illegible] s va. [illegible] tions sh
livered early. D [illegible] be [illegible] or 37
tion if one is con [illegible] ity [illegible] ere
signs of toxemia, t [illegible] te [illegible] o
The mode of de [illegible] n
degree of parity, [illegible] ,
Wherever feasible,

utilization of such procedures as stripping the membranes, rupture of the membranes when the cervix is favorable, and judiciously using oxytocin for induction. Delivery is preferably accomplished under caudal or other regional anesthesia.

Hormones

Smith and Smith and others demonstrated that patients with accidents in late pregnancy, and particularly diabetic patients, have abnormal hormone patterns, with high serum gonadotropin levels and low serum and urine estrogen and urine pregnanediol levels. Attention has since been centered on the correction of these abnormalities by administration of estrogen alone or with progesterone, but the efficacy of hormone therapy in toxemia and in loss of the fetus in late pregnancy remains controversial.

Priscilla White, who reports the largest experience with pregnant diabetics with the best results, attributes an appreciable share of her success to parenteral administration of hormones. Presently she administers parenteral estrogen and parenteral progesterone in increasing doses throughout pregnancy to that majority of her patients exhibiting any evidence of hormonal imbalance, as measured by serum gonadotrophin and urinary pregnanediol levels. Others have attributed her success more to the scrupulous care she gives her patients throughout pregnancy and her sagacious handling of those factors previously mentioned. In an effort to solve the controversy, a co-operative study was undertaken in England where, insofar as possible, alternate patients were treated with and without hormones. Although the fetal survival rate was not striking in either group, it was almost precisely the same in both groups. From these studies the inference is clear that hormone administration has no effect on the outcome of pregnancy in the diabetic. This view has been accepted by many workers in the field. White questions these interpretations of the results on the grounds that there was no selection of patients and that, in her opinion, the types of hormones administered were not comparable and were not given parenterally. Thus, it would appear that the validity of the use of hormones in pregnancy remains in question and cannot be unequivocally answered at this time.

OUTCOME OF PREGNANCY

Care of the Child

Proper attention to the previously mentioned factors enhances the status of the child at birth and makes neonatal care less difficult. Regardless of birth weight or time of delivery, the child born of the dia-

betic mother should be treated as premature. The contents of the respiratory passages and stomach should be carefully aspirated. The infant should have posturdal drainage in an incubator and his position should be changed frequently. High humidity is desirable; oxygen *should be used only as needed and, if the infant's condition permits,* should never be raised above 35 to 40 per cent, to avoid retrolental fibroplasia. If there is any evidence of respiratory distress, antibiotics should be employed. No fluids should be given by mouth for at least 48 hours, both to dehydrate the infant and to prevent possible aspiration. Then oral glucose is given, but no sodium-containing fluids are permitted for at least 72 hours. Standard infant feedings are then instituted. Although hypoglycemia frequently occurs in the first few hours after birth, this also is present in the infants of normal pregnancies and rarely, if ever, is a factor in neonatal mortality.

Hyaline membrane disease is to be feared in these babies. The use of measures mentioned above may lessen this hazard. Caesarean section is associated with an increased incidence of this complication and, where possible, should be avoided. Rigid sodium restriction and use of chlorothiazide in the mother has definitely lessened the incidence of respiratory difficulty and edema in these infants.

In spite of all these measures, the child of the diabetic mother is often edematous, particularly if allowed to approach full term. After delivery, sodium diuresis and potassium retention may occur in the infant, and he may have an increased excretion of 17-hydroxycorticosteroids and 17-ketosteroids.

The infant of the diabetic mother often exhibits splanchnomegaly, especially of the heart and reticuloendothelial system. Excess growth hormone has been implicated in this organ enlargement. Extramedullary hematopoiesis is frequently present. There are also hyperplasia and hypertrophy of the islets of Langerhans. Presumably, the latter is the result of elevated maternal and fetal blood sugars in utero.

Congenital defects have been noted to be about six times more common in the infants of diabetic women than in those born of normal women.

Diabetes itself is frequent in children of diabetic mothers. White found clinical diabetes in 9 per cent of 105 children born of diabetic mothers, and chemical diabetes (by glucose tolerance test) in an additional 14 per cent. Interestingly enough, this incidence was comparable in children born of diabetic fathers, being clinical in 9 per cent and chemical in 12 per cent. Where both parents were diabetic at the time of the baby's birth, clinical diabetes occurred in 32 per cent and chemical diabetes was present in an additional 30 per cent.

Mortality

With proper attention to the care of both the mother and the fetus, the prospects for producing a living child are fairly good. The maternal mortality should be very low. In the United States the average is 0.7 per cent. The fetal mortality ranges from 10 per cent to considerably higher, depending upon the type of patient seen, including age, the duration of diabetes, and the presence of complications. In 1947, Henley reviewed the world literature and noted a fetal mortality of 36.6 per cent in 1,269 cases. Generally, with the type of care available to most patients in the diabetes clinics of this country, the mortality is now about 20 per cent. Although the incidence of congenital malformations is universally high, this appears to have very little bearing on the actual fetal mortality.

ADVISABILITY OF PREGNANCY IN THE DIABETIC

In concluding the discussion on management of the pregnant diabetic, consideration of the wisdom of allowing pregnancy in the presence of diabetes seems in order. Since pregnancy is a strain on the woman who has had the disease for a long period of time, it would appear to be a wise policy to advise tubal ligation after she has attained two living children. Pregnancy is not advocated in the presence of extensive calcification of the pelvic arteries, proliferative retinitis, or nephropathy to the point of incipient renal failure, as evidenced by nitrogen retention. Interruption of pregnancy must be seriously considered in the presence of these complications.

Also to be considered is the advisability of pregnancy in the face of high fetal mortality, the frequency of congential abnormalities, and the incidence of diabetes in the children born of diabetic women. On the other hand, one faces a woman who may well have lived with diabetes for many years, whose life has been comprised of denials. Who is to deny her the right to have children? It is probably best to present all the facts to the patient and let her make her own choice. Human nature being what it is, the choice is almost invariably in favor of pregnancy.

SUMMARY

Pregnancy in the diabetic presents a complex medical, obstetric, and pediatric problem. It is accompanied by an increased susceptibility to infection and acidosis and an increased incidence of toxemia and hydramnios. Careful regulation of the maternal diabetes, prevention and treatment of toxemia and hydramnios, and a wise choice of the

time and technique for delivery, plus good neonatal care are imperative if the excessive fetal mortality associated with pregnancy in the diabetic is to be avoided. The wisdom of allowing pregnancy in diabetics with nephropathy, extensive atherosclerosis, or retinopathy is discussed and termination of pregnancy in the presence of these complications is considered.

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Chapter 45

INTERRELATIONS OF DIABETES AND DISEASES OF THE LIVER, THYROID, GASTROINTESTINES, AND CEREBRUM

Joseph L. Izzo

INTERRELATIONS OF DIABETES AND DISEASES OF THE LIVER

Role of the Liver in Blood Glucose Homeostasis

Before discussing the interrelations of diabetes and hepatic dysfunction, it may be worthwhile to review briefly the physiologic role of the liver in regulation of blood glucose and the major factors involved in formation and breakdown of liver glycogen, the *chief source* of glucose for tissue needs during fasting. Various aspects of the subject have been considered in detail elsewhere in this book, but are summarized here in order to reorient the reader to the discussion that follows.

BIOCHEMICAL ASPECTS. The liver is the only organ which, from a practical standpoint, is capable not only of removing glucose from the blood, storing it temporarily as glycogen, and releasing it to the blood *again as glucose upon tissue demand*, but also *of forming glucose from* noncarbohydrate sources, such as amino acids, in quantities sufficient

to meet bodily needs. Kidneys have some capacity for gluconeogenesis and maintenance of blood glucose, but this is trivial compared with that of the liver (52). Other peripheral tissues, such as skeletal muscles, are unable to release glucose into the blood, owing to lack of the enzyme

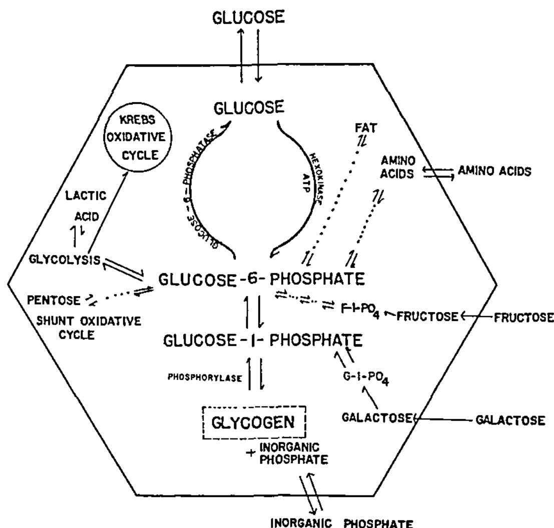


FIG. 45-1. Simplified diagrammatic representation of liver cell showing the main pathways of metabolism, the main sources of glycogen, and some of the more important steps involved in its formation and degradation.

glucose-6-phosphatase. Consequently, the needs of tissues for glucose during fasting are met largely by the liver.

Schematically outlined in the simplified diagram in Figure 45-1 are the main sources of hepatic glycogen and some of the more important steps involved in its formation and degradation. In addition to glucose and other hexoses such as fructose and galactose, many of the amino

acids serve as precursors for glycogen via the pathways shown in Figure 45-1. Glycerol from fat, glycolytic products such as lactic and pyruvic acids, and a variety of quantitatively less important substances can also serve as precursors for glycogen. The first step in processing of glucose after it enters the hepatic cell is the formation of glucose-6-phosphate from the reaction of glucose with adenosine triphosphate (ATP) in the presence of hexokinase. This serves to "capture" the glucose for subsequent metabolic reactions since, unlike free glucose, phosphorylated glucose is relatively unable to penetrate the cell membrane and therefore is "locked" within the cell. Glucose-6-phosphate can now be converted to glycogen after first being transformed to glucose-1-phosphate;* or it can be metabolized either via the Embden-Meyerhof glycolytic pathway or via the so-called pentose shunt pathway. The latter pathway is oxidative; the former can produce lactic acid or can couple to the Krebs oxidative cycle, presumably through the transfer of pyruvate to the mitochondria without the formation of lactate. Glucose-6-phosphate can also be dephosphorylated by the enzyme glucose-6-phosphatase to form glucose again, which is free to leave the cell.

Note in Figure 45-1 that glycogen forms a *cul-de-sac* off the main pathways of glucose metabolism, glycogenolysis proceeding by a direct reversal of the biochemical steps involved in glycogenesis.† Glycogen may be considered to represent a glucose reservoir that increases or decreases in size, depending upon availability of substrate on the one hand and energy needs of hepatic as well as extrahepatic tissues on the other. The extent of formation of glucose from nonglucose precursors (gluconeogenesis) will vary in accordance with the relative rates of entry and exit of glucose into and from the liver cells. In other words, more glucose will be formed from nonglucose precursors when less glucose enters or more glucose leaves the cell and, conversely, less glucose will be formed from nonglucose precursors when more glucose enters or less glucose leaves the hepatic cell. It should be apparent that the level of glycogen does not necessarily reflect the rate of glucose metabolism in the cell.

PHYSIOLOGIC ASPECTS. The fasting blood sugar is maintained relatively constant by a dynamic balance between the rate of hepatic glucose

* Since this chapter was written, evidence has been accumulating supporting the findings of Leloir and Cardini (*J. Am Chem Soc.* 79:6340, 1957) and suggesting that the main pathway for the synthesis of glycogen may involve uridine diphosphate glucose (UDPG) rather than glucose-1-phosphate (Villar-Palasi, C *Fed. Proc.* 18:344, 1959, Breckenridge, B. M., and Crawford, E J. *Ibid.* 18:197, 1959).

† Synthesis of glycogen via UDPG pathway instead of via glucose-1-phosphate would not invalidate the concept that glycogen forms a *cul-de-sac* off the main pathway of glucose metabolism.

output and the rate of utilization of glucose by muscle and other peripheral tissues. Since the blood is cleared of ingested glucose in about two hours, it is evident that the liver must supply glucose to the circulation for as many as eighteen hours of the day.

In fasting normal humans the mean rate of net splanchnic* glucose production as measured by hepatic vein catheterization technique has been estimated by Bondy, *et al.*, as 3.5 mg./kg./min., or 115 mg./sq. M. of body surface/min., and by Myers as 2.0 mg./kg./min., or 65 mg./sq. M. of body surface/min. This means that the liver of a 70-kg. man would supply glucose to the blood at a mean basal rate of 8 to 15 gm. per hour during fasting. As pointed out by Bondy, the stores of hepatic glycogen would hardly be sufficient to maintain this rate throughout the overnight fasting period of 12 hours or more, since the content of glycogen in the postprandial liver probably does not exceed 6 per cent of the weight of the organ (21), or, in other words, 90 gm. in a liver weighing 1500 gm. Consequently, the conversion of amino acids and other precursors to glucose is a necessary step in maintaining the blood sugar at physiologic levels. Actually, glycogenolysis is not restricted to periods of fasting. Liver glycogen is continuously being formed and broken down at a fairly rapid rate and is in a dynamic steady state even when the quantity of glycogen in the liver is maintained at fairly uniform levels (62).

HORMONAL ASPECTS. Although the hepatic production of glucose is governed basically by the level of blood sugar itself, several hormones directly or indirectly affect this process. Epinephrine, from the adrenal medulla, and glucagon, presumably from the α-cells of the pancreatic islets, enhance hepatic glucose output. Recent experimental studies (3, 51) have suggested that insulin, on the other hand, inhibits the release of glucose by the liver in addition to accelerating the removal of glucose by peripheral tissues. This dual action of insulin is supposed, by some investigators (51), to be accomplished by a binding of insulin at the cell surfaces in such a way that the inward flow of sugars is favored and the outward flow prevented. This could explain why the diabetic secretes glucose into the blood in the face of hyperglycemia. However, such an effect of insulin on the liver has been challenged by the very recent studies of Mahler, *et al.*, who found that the decrease in blood sugar in dogs associated with insulin infusion was due entirely to in-

* As used in this chapter, the term splanchnic glucose production refers to hepatic glucose production plus the glucose produced by the viscera drained by the portal vein. Since the contribution of the latter to the total glucose production from the splanchnic area is small, one may assume that the calculated net splanchnic glucose production approximates closely the true hepatic glucose output (45).

creased utilization of glucose in tissues including the gut and was not in any way due to a decrease in hepatic glucose production.

The hyperglycemic actions of epinephrine and glucagon are associated with a rapid breakdown and depletion of liver glycogen in the well-nourished animal. Epinephrine also exerts a peripheral inhibitory action on glucose utilization, resulting in increased quantities of lactic and pyruvic acids in the blood which, in turn, are recycled through the liver to form new glucose (Cori cycle). A peripheral action of glucagon has not been established. However, unlike epinephrine, glucagon in large amounts has a marked action in causing protein catabolism in man and in rats, which is probably brought about by increased hepatic gluconeogenesis from amino acids and is not dependent upon but is enhanced by the presence of the adrenal cortex (27, 28). This suggests a possible key role of glucagon in physiologic blood glucose homeostasis that merits detailed study.

The modes of action of both epinephrine and glucagon are purported to involve the conversion of phosphorylase B, the inactive form of the enzyme phosphorylase that is involved in glycogen formation and breakdown (see Fig. 45-1), to phosphorylase A, the active form (62). However, on the basis of the evidence available at the present time, it is difficult to see how such a mechanism could satisfactorily explain all the physiologic effects of glucagon and epinephrine.

Excessive administration of thyroid hormone can also bring about a mobilization and disappearance of liver glycogen by mechanisms that are not understood at present (62).

The 11-oxysteroids of the adrenal cortex tend to promote the accumulation of liver glycogen. This is due, at least in part, to an augmented production of glucose from glycogenic amino acids, a process possibly secondary to a stimulation of protein mobilization. The physiologic importance of this action is demonstrated by the difficulty encountered in maintaining a normal fasting blood glucose level in hypoadrenal states where hypoglycemia is a frequent complication.

In most species including man a deficient supply of insulin results in a diminished store of hepatic glycogen, which is associated with a continuing release of glucose to the blood *in spite of a mounting level of sugar in the blood.* The latter phenomenon has never been explained in a satisfactory manner. Administration of insulin restores hepatic glycogen to normal levels; but at least in some species, excessive amounts of insulin do not increase the content of hepatic glycogen above normal (62). A possible mode of action of insulin in influencing hepatic glycogen stores directly has been mentioned above.

NEUROREGULATORY FACTORS. Hepatic glycogenesis-glycogenolysis is

under neural as well as endocrine influence. The sympathetic division of the autonomic nervous system regulates hepatic glycogenolysis directly via the splanchnic nerves to the liver and also indirectly by stimulating secretion of epinephrine by the adrenal medulla. This is discussed in more detail in the section on diabetes and the cerebrum (see below).

OTHER FACTORS. In addition to neurologic and endocrine influences, several other factors affect the quantity of liver glycogen. Animals maintained on carbohydrate-poor diets have less glycogen in their livers than those maintained on high carbohydrate diets. Starvation results in a very marked depletion of liver glycogen in 24 hours. Conditions that impose a drain upon body reserves, such as violent exercise, also result in reduced hepatic glycogen stores. Experimental poisoning with phlorhizin, a procedure which lowers the renal threshold for glucose, has a similar effect. Anoxia and acidosis also induce similar effects by mechanisms that are not clear at present (62).

Disturbances in Blood Glucose Homeostasis Associated with Diseases of the Liver

In view of the central role of the liver in blood glucose regulation it is not surprising that abnormalities in carbohydrate tolerance have been observed in acute and chronic diseases of the liver accompanied by failure of hepatic function. The remarkable feature is not that changes do occur but rather that they occur relatively infrequently and in most cases are mild, thus attesting to the tremendous reserve power of the liver in respect to carbohydrate metabolism. Spontaneous hypoglycemia and/or postprandial glycosuria are known to occur in cases of acute ascending cholangiolitis, toxic or infectious hepatitis, acute yellow atrophy, hepatic carcinomatosis, fatty metamorphosis, passive congestion, and various types of cirrhoses. In acute reversible liver disease changes in carbohydrate metabolism are usually temporary, and presumably reflect generalized moderate damage to hepatic cells, which tends to disappear with healing. Of graver import are the changes brought about by chronic diseases that result in destruction of large portions of the organ, as in Laennec's cirrhosis and posthepatitis necrotic cirrhosis.

In hepatic disease accompanied by impairment of glycogenic function of the liver, the oral dextrose tolerance test indicates a delay in clearing the blood of absorbed sugar. Peak values for blood sugar at 30 to 60 minutes may be abnormally high, or a high plateau type of curve similar to that seen in diabetes mellitus may occur. The feature distinguishing between the abnormal glucose tolerance curves related to liver dis-

ease and to mild diabetes is said to be the fasting blood sugar. In liver disease, fasting blood sugar tends to be lower than normal, whereas in diabetes it is higher than normal.

The derangements in metabolism of glucose characteristic of liver disease are illustrated by the following case reported by Conn (Fig. 45-2):

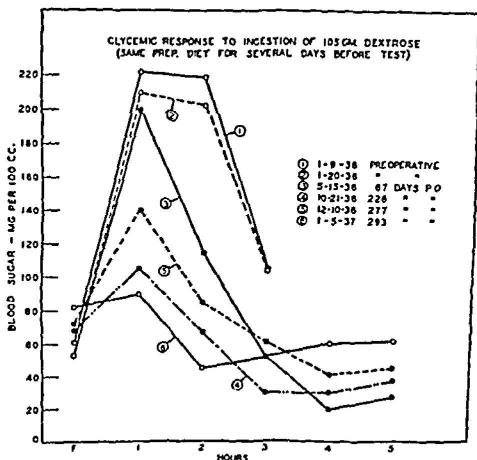


Fig. 45-2. Case of hepatogenic hypoglycemia: Dextrose tolerance curves before and after cholecystectomy (From Conn, J W.: *J.A.M.A. 115:*1669, 1940.)

A 47-year-old laborer experienced hypoglycemic episodes at night, which were relieved promptly by dextrose. A glucose tolerance test revealed a diabetic type of curve except for a low level of fasting blood sugar. Laboratory studies showed evidence of hepatic dysfunction and nonfunctioning gallbladder. Surgical exploration of the abdomen was done and a cholescystectomy performed for suppurative cholecystitis and biliary cirrhosis. The hypoglycemic symptoms disappeared after surgery and the glucose tolerance reverted to normal.

Lande and Pollack also observed hyperglycemia and glycosuria associated with biliary tract disease. Disturbances in carbohydrate metabolism were directly correlated with the degree of impairment of hepatic function. Restoration of liver function to normal by adequate biliary drainage was accompanied by disappearance of the diabetic manifestations and return of the glucose tolerance to normal.

It should be kept in mind, however, that while low fasting blood glucose levels (hypoglycemia) coupled with a high plateau type of tolerance curve strongly suggest hepatogenic diabetes, this situation is uncommon. Fasting hypoglycemia is seen only rarely in the majority of cases of liver disease (65), except for hepatic carcinoma, where the incidence of fasting hypoglycemia is reported as fairly common (41) for reasons that are not well understood. In cases of liver disease other than cancer, the fasting blood glucose concentration is usually normal, but in some cases may be slightly elevated. Thus, in many instances the abnormal glucose tolerance curves in liver disease following a glucose load are indistinguishable in type from those obtained in mild degrees of diabetes mellitus. To add to the difficulty in distinguishing between these two conditions from the glucose tolerance curves, many patients with liver disease, such as the alcoholic with cirrhosis, are anorectic and malnourished so that the abnormalities include the characteristics of "starvation diabetes." The correct interpretation of oral dextrose tolerance tests also can be questioned, because these tests fail to take into account abnormalities of absorption that may occur in this type of liver disease. However, glucose loads administered intravenously also reveal some delay in clearance of the administered glucose from the blood in a considerable number of patients with cirrhosis (7). The impairment is frequently so mild that glycosuria is often absent. Impairments in the processing of fructose and galactose can also occur in hepatic disease, with a resultant delay in clearance of these sugars from the blood (7). Normally, these hexoses are rapidly converted to glycogen or glucose-6-phosphate in the liver.

Low liver glycogen secondary to an impaired ability of the damaged liver to trap glucose and convert it to glycogen has been generally accepted as the primary basis for the abnormalities in blood glucose homeostasis seen in extensive hepatic disease. Several techniques have been used to measure indirectly possible differences, in comparison with normals, in hepatic glycogen stores in patients with hepatic disease. Patients with hepatic disease have repeatedly shown a lesser rise in blood glucose than normal individuals following administration of glycogenolytic agents such as epinephrine and glucagon (13, 33, 59). Measurements of hepatic glucose output by the technique of catheteri-

zation of the hepatic vein during basal fasting state and following the administration of glucagon have yielded lower values in cirrhotics than in normals (46). Also, administration of phlorhizin to patients with hepatic dysfunction has resulted in a fall in blood sugar and development of ketosis, whereas in normals the blood sugar remained relatively constant and ketosis did not develop (34). Furthermore, the patients with hepatic disease excreted less glucose in the urine under such conditions than normals. In general, the results with all these techniques have been consistent with the hypothesis that diseased livers release less glycogen than normal livers, presumably because of poor stores. However, direct measurements of liver glycogen in hepatic disease by liver biopsy technique have been few and inconclusive (37). In some cases of liver diseases, cirrhosis for example, reduced stores of glycogen can vary, apparently, not necessarily because of a decreased concentration of glycogen in each cell but rather because of a reduced total cell mass, since in cirrhosis the concentration of glycogen in liver biopsy specimens has not been found significantly lower than in normals, when areas of fibrosis are excluded (7).

An additional factor in advanced cirrhosis leading to decreased release of hepatic glycogen is the reduction in splanchnic blood flow (45). This can account, at least in part, for the diminished splanchnic glucose production in cirrhosis (Table 45-1). Reduced blood flow can result in two ways: first, by the great distortion in the vascular pattern as the regenerating nodule of cirrhosis develops, resulting in a crowding of the vessels in the dense, barren connective tissue septa with relative avascularity of the nodule; and second, by the development of anastomoses between portal and hepatic veins, with further shunting of portal blood from the hepatic parenchyma (40).

Elevations in fasting blood sugar that are seen in some cases of hepatic disease cannot be explained on the basis of a decreased content of glycogen in the liver. Neither can the diminished hypoglycemic response to insulin observed in some cases of liver disease be explained on such a basis (13). In both cases low stores of glycogen should lead to blood levels less than those obtained in normals. In this respect the studies of Lang and his associates on glucose uptake of peripheral tissues of dogs with and without the presence of the liver are of interest. Their findings suggest that the liver releases some "humoral agent or influence" that promotes uptake of excess glucose by peripheral tissues. Such an influence of the liver could explain both the high fasting blood sugar and the reduced glucose tolerance often observed in liver disease. Recent experimental findings of Schwartz and Mertz strengthen such a concept. A glucose tolerance factor (GTF) has been

TABLE 45-1. NET SPLANCHNIC GLUCOSE PRODUCTION IN NORMAL AND DISEASED SUBJECTS: MEAN DATA*

Group	*Number in group*	*Oxygen consumption as percentage of normal basal (Per cent)*	*Hepatic blood flow (Ml. per min. per sq. M.)*	*Splanchnic oxygen consumption as percentage of normal*† *(Per cent)*	*Arterial blood glucose conc. (Mg. per cent)*	*Hepatic A-V glucose diff. (Mg. per cent)*	*Net splanchnic glucose production (Ml. per min. per sq. M.)*
Controls	38	106 ± 2	764 ± 25	95 ± 3	95.9 ± 1.6	8.5 ± 0.1	64.6 ± 3.7
Convalescent and chronic infections	8	106 ± 5	806 ± 76	105 ± 6	101.6 ± 5.3	9.7 ± 1.0 p = 0.2	79.6 ± 11.2 p > 0.1
Diabetes mellitus, insulin treated	9	110 ± 5	781 ± 71	111 ± 8	177.9 ± 27.9	9.6 ± 0.9 p > 0.2	70.7 ± 3.8 p > 0.4
Laennec's cirrhosis	18	108 ± 4	695 ± 46	89 ± 5	92.9 ± 2.6	7.2 ± 0.5 p > 0.05, <0.1	49.1 ± 3.6 p < 0.02
Hyperthyroidism	10	148 ± 7	865 ± 55	153 ± 7	101.7 ± 1.3	8.7 ± 0.6 p > 0.5	71.8 ± 7.1 p = 0.2
Congestive heart failure	8	116 ± 7	450 ± 40	114 ± 13	88.3 ± 2.5	11.5 ± 1.0 p < 0.01	30.1 ± 1.3 p > 0.05, <0.1

* From J. D. Meyers (45).

† The normal splanchnic oxygen consumption in a larger series of 48 controls was 34 ml. per min. per sq. M. This value is taken as 100 per cent.

isolated from the livers of rats, which prevents or corrects the impairment of glucose removal by the periphery in rats fed diets that produce liver necrosis.

From a consideration of the available clinical and experimental evidence it seems that the abnormalities in the homeostasis of blood glucose may stem from multiple factors acting in various combinations. In addition to a reduced glycogen content, other factors such as impaired effective hepatic blood flow in special types of hepatic disease and as yet unidentified hepatic factors regulating peripheral glucose uptake may also be involved. Furthermore, the effects of variable alterations in the functional levels of specific enzymes as well as hormonal influences needs to be further explored.

In addition to the diseases of the liver just described, derangements in hepatic glycogenesis-glycogenolysis owing to inborn errors of metabolism also lead to disturbances in blood glucose homeostasis. Glycogenesis in the absence of glycogenolysis (Von Gierke's disease) is a relatively rare inborn error of metabolism characterized by massive increases in hepatic glycogen, up to 15 per cent of the net weight of the organ, and at the same time by an impaired ability to release glucose to the blood. The defect appears to be caused by an impediment in the breakdown of liver glycogen to glucose. In some cases this has been attributed to a deficiency in the hydrolytic enzyme glucose-6-phosphatase, and in other cases to a deficiency of the so-called branching enzyme. Lack of the latter enzyme causes structural differences in the degree of branching of the abnormal glycogen as compared to normal glycogen (62).

Differentiation of Diabetes Mellitus with Secondary Liver Disease from Hepatogenic Diabetes

It is apparent from the foregoing discussion that the differentiation of mild diabetes mellitus with secondary liver disease from hepatic glycogenic dysfunction secondary to liver disease frequently poses diagnostic problems. As pointed out above, the glucose tolerance test does not always distinguish between these two conditions. Similar difficulties have been encountered with the use of other tests, such as the histochemical determination of hepatic glycogen content, galactose tolerance, fructose tolerance, the response of the levels of blood glucose, lactic and pyruvic acids to the administration of epinephrine or glucagon, and finally studies of plasma lipid and cholesterol levels (37). On the other hand, determination of the changes in serum inorganic phosphorus during the intravenous glucose tolerance test has been advocated as a useful adjunct in distinguishing diabetes mellitus from

hepatic glycogenic dysfunction associated with liver disease. The test is based on the concept that the fall in inorganic serum phosphorus is related largely, if not entirely, to the active entry of glucose into the glycolytic cycle of peripheral tissues, especially skeletal muscle, and little, if any, to the entry of glucose into the glycogenic cycle in the liver. Experimental support for this concept comes from the studies of Bolliger and Hartman in which they showed a brisk fall in serum inorganic phosphorus when the isolated hind limb of a dog was perfused with glucose and phosphate, whereas no such fall occurred when the isolated liver was similarly perfused. Pollack, *et al.*, confirmed these results by showing that the phosphorus fall was still present in hepatectomized dog, whereas it was absent in the totally depancreatized dog. In fact, the rate and degree of phosphorus change was accentuated by hepatectomy.

Forsham and Thorn have used a standardized intravenous glucose tolerance test employing 0.5 gm. of glucose per kilogram of body weight in a 20 per cent solution administered over a 30-minute period. Blood sugar and serum inorganic phosphorus are determined at half-hourly intervals for 2 hours and once again after 3 hours. In a group of normal subjects a 25 per cent average fall in serum inorganic phosphorus occurred in 60 to 90 minutes after the beginning of the infusion. In the presence of hyperglycemia, patients with absolute or relative insulin lack showed only a 12 per cent fall, whereas patients with primary liver storage deficiency owing to various types of liver disease showed an average fall of 37 per cent (Fig. 45-3). These results were confirmed by Smith, *et al.*, who also obtained in hepatic disease a significantly greater fall in serum phosphorus associated with glucose infusions than in diabetes. However, as pointed out by Smith, the wide individual variations limit the diagnostic significance of this difference. The increased fall in serum phosphorus following glucose infusion in patients with hepatic glycogenic dysfunction is presumed to be due to a greater diversion of glucose to muscles because of the impaired ability of the liver to trap and store glucose.

An additional complicating factor in the interpretation of the blood glucose-phosphorus curves discussed in the preceding paragraph is the recent report by the author (26) describing a hitherto unrecognized reciprocal relationship between levels of fasting blood glucose and plasma inorganic phosphorus occurring in diabetics. In general, low levels of blood sugar tended to be associated with high levels of phosphorus and vice versa. A typical example of this relationship is shown in Figure 45-4. There appeared to be only a slight tendency, if any, toward a low positive correlation between the 24-hour urinary excretion

of glucose and phosphorus, suggesting that the observed phenomena reflect altered equilibria between phosphate stores and circulating phosphorus rather than alterations in the renal mechanism governing phosphate excretion. The significance of these findings remains to be established, but the observations are of interest since they are the reverse of

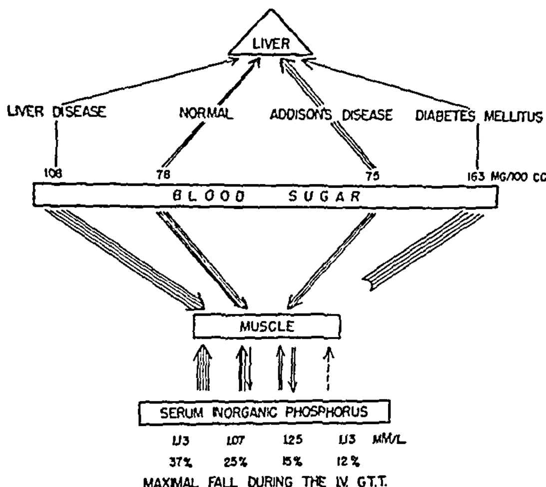


FIG. 45-3. Common patterns of glucose-phosphorus curves. Note that it is assumed that the level of serum inorganic phosphorus is regulated by its rate of entry as well as its rate of exit from muscle. (From Forsham, P. H., and Thorn, G. W.: *Proc. Am. Diabetic Assoc.* 9:99, 1949.)

the well-known short term changes in phosphorus caused by glucose administration. Although the observed phenomena are not strictly analogous to the short term studies just alluded to, it is conceivable that they might influence the phosphorus response to glucose in diabetics. This may in part explain the failure of some investigators to

find appreciable differences in the serum phosphorus responses of the diabetic and nondiabetic (14).

In the final analysis, the differential diagnosis between hepatic glycogenic dysfunction and diabetes mellitus may have to be made on clinical grounds, when the various laboratory tests give equivocal results. Leevy suggests the following criteria for a diagnosis of hepatogenic diabetes: (1) a history of exposure to hepatotoxins or liver injury

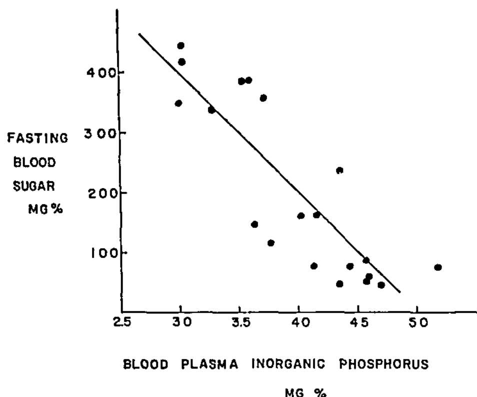


FIG. 45-4. Correlation between daily fasting blood sugar and plasma inorganic phosphorus in a female patient, 21 years old, with unstable diabetes mellitus treated by insulin and chemically constant diet in the metabolism ward. (From Izzo, J. L., Roncone, A. M., and Eilers, A.: *Proc. Soc. Exper. Biol. & Med.* 91:373, 1956.)

prior to the appearance of the diabetic syndrome; (2) physical, biochemical, or histologic evidence of liver disease (or all three types); (3) no familial history of diabetes or biochemical evidence of pancreatic, pituitary, adrenal, or thyroid disease; (4) disappearance of hyperglycemia and glycosuria without the administration of insulin, after treatment for hepatic disease.

Effect of Diabetes on the Liver

Hepatic enlargement in severe uncontrolled diabetes, especially during recovery from acidosis or coma, is well recognized. Before the advent of long-acting insulins, hepatomegaly was seen commonly in juvenile and also in some adult diabetics. With the introduction of long-acting insulins and better control of diabetes the incidence has decreased, but hepatic enlargement continues to be observed not infrequently, not only during recovery from acidosis or coma but occasionally even with fairly adequate regulation of the diabetes. Although the nature of the hepatic enlargement is not well understood, it seems to be due, at least in part, to increased accumulation of fat (31).

Some investigators have reported various abnormalities in hepatic function in diabetics as measured by the different routine liver function tests, while others employing the same battery of tests have found no evidence of hepatic dysfunction in the majority of cases (31). It would seem, on the basis of available evidence, that hepatic function is not grossly impaired in most diabetics, as measured by routine laboratory tests, and that in most of the instances where abnormal results were obtained, a definite relationship to diabetes has not been established.

Effect of Diseases of the Liver and Biliary Tract on Pre-existing Diabetes

Amelioration of the diabetic state has been reported to occur occasionally when cirrhosis is superimposed on pre-existing diabetes (49). Resistance to insulin in some cases of hemachromatosis after the development of cirrhosis of the liver is well known (31, 49, 63). Sudden resistance to insulin has also been observed in a diabetic patient following thrombosis of the hepatic artery (49).

Cholecystitis and cholelithiasis probably occur somewhat more frequently among diabetics than in nondiabetics, but a causal relationship has not been established (31).

INTERRELATIONS OF DIABETES AND DISEASES OF THE THYROID

Hyperthyroidism and Diabetes

EFFECTS OF HYPERTHYROIDISM ON CARBOHYDRATE METABOLISM. Disorders of carbohydrate metabolism in uncomplicated hyperthyroidism have been recognized for a long time. The clinical syndrome of alimentary hyperglycemia and glycosuria, weight loss, abnormalities in carbohydrate tolerance as judged by the oral dextrose tolerance tests, and tendency toward ketosis during relatively short periods of fasting

resemble superficially at least, the clinical manifestations of diabetes mellitus in many respects. Disturbances of carbohydrate metabolism occur frequently in hyperthyroidism, but in the majority of instances are relatively mild. In a series of 500 cases of thyroid disease, Joslin reported the incidence of glycosuria as follows: hyperthyroidism 38.6 per cent; adenomatous goiter with secondary hyperthyroidism 27.7 per cent; nontoxic goiter 14.8 per cent. The characteristic response to a standard glucose tolerance test using 100 gm. of dextrose by mouth is a curve starting at normal or only slightly elevated blood sugar values (up to 120 to 140 mg. per cent), rising to an abnormally high peak in 30 to 60 minutes with values frequently exceeding 200 mg. per cent, and a returning to normal values or below within 2 to 3 hours (63).

The diabetic-like response to ingested dextrose in hyperthyroidism is probably related to the increased rate of absorption of glucose from the intestinal tract and to the increased rate of hepatic glycogenolysis in the presence of excessive quantities of thyroid hormone rather than to a diminished rate of utilization of glucose by the extrahepatic tissues. In fact, the available evidence indicates that the utilization of glucose by the extrahepatic tissues may actually be increased.

Evidence in favor of increased utilization of glucose by the peripheral tissues in hyperthyroidism is as follows:

1. The respiratory quotient in cases of hyperthyroidism not only rises as in normals after a test meal of glucose, but the rise is more abrupt. This is in contrast to the response in true diabetes, where the rise of the quotient is usually sluggish and in some cases lacking altogether (63).

2. Direct studies of the action of thyroxine on tissues have revealed that there is an increased uptake of oxygen (15).

3. The difference between arterial and venous glucose concentrations is greater in individuals with hyperthyroidism than in normals (9).

4. Studies of eviscerated rabbits with and without pretreatment with thyroid hormone have shown that the extrahepatic tissues of the hyperthyroid rabbit remove glucose from the blood at a much greater rate than do those of the normal animal (42).

5. Studies in man on the rate of removal of glucose from the blood following the intravenous administration of a glucose load have revealed that in hyperthyroidism the rate of disappearance of glucose from the blood tends to be either normal or increased (2). In some cases with normal or increased glucose disappearance, the oral glucose tolerance curves were interpreted as diabetic in type. Differences in rates of removal of blood glucose in a patient during the hyperthyroid and euthyroid states are shown in Figure 45-5.

betes, which previously had been easily and well regulated by means of an 1,800-calorie diet and the administration of 45 to 50 units of insulin daily. During the week prior to admission, the urine samples had shown consistently 4+ sugar and variable amounts of acetone, even though the insulin dose had been increased to 70 units daily. Physical examination revealed tachycardia and an enlarged thyroid with audible bruit but no tremor or exophthalmos. Marked glycosuria and ketonuria were noted on urinalysis. The basal metabolic rate, serum protein-bound iodine, and radioactive iodine uptake by the thyroid were consistent with the clinical diagnosis of *diffuse goiter with hyperthyroidism*. Tapazole was started and the diabetes was brought under control by the administration of 110 to 120 units of insulin per day. The patient was discharged 10 days later after being placed on the following regime: a 2,600-calorie diet, 40 mg. of Tapazole daily, and 100 units of insulin daily.

Following discharge from the hospital, the patient showed progressive improvement and by the end of the month showed euthyroid function by clinical and laboratory criteria. The insulin requirements had declined progressively from 100 units to 45 to 50 units daily. The dose of Tapazole was maintained at 40 mg. per day. However, during the subsequent 10 months she began to experience unusual sensitivity to cold, complained of being mentally and physically sluggish, and developed localized puffiness about the eyes, hands, and anterior surfaces of the lower extremities. The insulin requirement gradually diminished to 35 units per day. On gradual reduction of Tapazole to 10 mg daily and addition of Lugol's solution and small doses of thyroid extract, the latter symptoms gradually cleared. The insulin requirements again returned to 45 to 50 units daily. Tapazole was discontinued at the end of 9 months but was resumed 2 months later because of recurrent mild thyrotoxicosis. One of the first signs of recurrent thyrotoxicosis was a slowly increasing insulin requirement. A subtotal thyroidectomy was performed in March, 1957. Since the surgery, the patient has remained in a euthyroid state and her insulin requirements have been relatively stable at 45 to 50 units per day.

The fact that the requirements for exogenous insulin are greatly increased when hyperthyroidism is superimposed upon pre-existing diabetes suggests that in the nondiabetic, hyperthyroidism indirectly stimulates the pancreas to produce more insulin commensurate with the increased rate of metabolism. This concept receives support from experimental studies in which continued injections of thyroxin were administered to partially depancreatized animals (24) and also to animals that had previously been treated with subdiabetogenic doses of alloxan (43). The pancreatic islets of such animals invariably revealed evidence of islet damage, suggesting that the diabetes which developed in such animals represented exhaustion of the islets.

The mechanisms that bring out increased demand for insulin in

thyrotoxicosis are not clearly understood. Many workers in the field are of the opinion that the increased demand for insulin is nonspecific in nature and probably related to the increased metabolic rate (14). The increased rate of absorption of glucose from the gastrointestinal tract and increased rate of glycogenolysis and gluconeogenesis can conceivably increase insulin output by the pancreas by increasing the amount of glucose presented to the blood. Antagonism to insulin and increased rate of insulin destruction by an effect of thyroxin upon the insulinase-antiinsulinase system (14) have also been suggested as possible mechanisms.

The response to a test dose of insulin in the rabbit receiving thyroid hormone may be diminished or enhanced depending upon such factors as duration of thyroid feeding and changes in content of liver glycogen (40). Variations in response to epinephrine and in the content of liver amine oxidase also may influence the results (58). The latter enzyme can destroy epinephrine, which would otherwise antagonize insulin. In the beginning, thyroid feeding can diminish the hypoglycemic response to insulin, probably as a result of increased hepatic glycogenolysis, but later on as the liver glycogen stores become depleted the response to insulin may be augmented. Increased hypoglycemic response to insulin, which has been observed in animals and also in patients with hyperthyroidism, argues against any direct antagonism between insulin and thyroid hormone.

ROLE OF THE THYROID AS A CAUSATIVE FACTOR IN DIABETES. The increased need for insulin in diabetes complicated by thyrotoxicosis raises the possibility that the thyroid might play a primary role in development of diabetes similar to that of the anterior pituitary and adrenal cortex. However, neither experimental nor clinical evidence bears out this idea. The feeding of large amounts of thyroid hormone has not produced diabetes in animals with intact pancreas. Houssay was able to produce diabetes in animals fed thyroid extract (metathyroid diabetes) only when the pancreatic reserve had been drastically reduced by previous removal of most of the pancreas. It should be noted that the combination of diabetes and hyperthyroidism was found in only 1 per cent of a series of 32,148 diabetics from the Joslin Clinic (31).

The incidence of diabetes as a complication in a series of 3,471 cases of hyperthyroidism seen at the Mayo Clinic during a 3-year period from 1935 to 1938 was 3.3 per cent (63). In the cases with toxic nodular goiter, the incidence of diabetes was 5.7 per cent, whereas in the cases of diffuse toxic goiter it was only 1.7 per cent. The latter incidence was no greater than that for the frequency of diabetes among all new registrations at the Clinic in 1937 (1.8 per cent). Increased incidence

of diabetes in the group with toxic nodular goiter was attributed t usually long duration of this type of hyperthyroidism. As a result pancreas could be exposed to excessive demands for insulin ov longer period and thus be more likely to exhaust its insulin res However, it should be pointed out that toxic nodular goiter is monly seen in older patients in whom incidence of diabetes is greater. Attempts to obtain information bearing on the question of relationship of hyperthyroidism to diabetes by determining the quency of prior appearance of hyperthyroidism in cases in which perthyroidism and diabetes are associated have not been very frui There is a slight tendency for hyperthyroidism to appear before betes, but owing to difficulties in determining clinically with certa which disease appears first, the data are difficult to evaluate. From data presented it seems unlikely that hyperthyroidism is an impor factor in development of diabetes unless the predisposition to diab is already present.

DIAGNOSIS OF ASSOCIATED DIABETES AND HYPERTHYROIDISM. In vie the frequency of transient alimentary glycosuria and mild fasting perglycemia in uncomplicated hyperthyroidism, the diagnosis of diabetes associated with hyperthyroidism may present difficulties. magnitude of the problem is brought out by a survey of 620 hy thyroid patients with hyperglycemia carried out by John. After tr ment of the hyperthyroidism, the blood sugar reverted to normal in per cent of the cases, but in 30 per cent the hyperglycemia persis In order to avoid erroneous diagnosis of diabetes some authorities (63) have recommended that the upper limits of normality of bl sugar in hyperthyroidism be placed at 150 mg. per cent fasting, inst of the standard 130 mg.; and at 200 mg. per cent after a meal inst of the standard 170 mg. Even with these new criteria the diagn may be in doubt. In John's series, follow-up studies show that in so cases even a high blood sugar response following the ingested dextr might quickly revert to normal following treatment of the hyp thyroidism, whereas in others a less abnormally high response mi still remain above normal after therapy. Although sufficient experie is not available at present, it would seem that determination of rate of removal of glucose from the blood following a glucose load travenously, as employed by Amatuzio, *et al.*, might be a promisi laboratory tool for differentiating between diabetes and hyp thyroidism. In the latter disorder the removal rate is usually normal increased but occasionally is decreased, whereas in diabetes it is t rule that the rate of removal is decreased. In the final analysis a po tive diagnosis in doubtful cases may have to wait until the hyp

explanation for both findings is that the absorption of glucose from the gastrointestinal tract and removal from the blood are both delayed, owing to the retarded metabolic rate. Experimental studies have shown that the rates of absorption of hexoses from the gastrointestinal tract are markedly reduced in thyroidectomized rats (1).

Response to insulin may be either normal or increased in thyroidectomized animals and in myxedematous patients (61). Also, the insulin tolerance tests tend to show a delay in fall as well as a delay in return to normal of the blood sugar level, presumably owing to a general slowing of the corrective response to hypoglycemia (61).

EFFECTS OF HYPOTHYROIDISM IN DIABETES. Compared to depancreatized controls, experimental animals thyroidectomized both before and simultaneously with pancreatectomy excrete less quantities of glucose and nitrogen (16). However, the decreases are much less than those produced by removal either of the pituitary or adrenal glands in pancreatectomized animals. In man, development of spontaneous myxedema lessens the severity of the diabetic and reduces the insulin requirement (31). Thyroidectomy has been reputed to arrest or cure pre-exisiting diabetes on occasion, but some of the apparent returns to normal of sugar tolerance may be due to the development of myxedema, particularly in cases of exophthalmic goiter complicated by thyroiditis (63). Occasionally, diabetes may develop subsequent to an operation for hyperthyroidism. Spontaneous myxedema and diabetes occur together only rarely. In Joslin's series of 32,148 diabetics, myxedema was recognized in only 11 cases, of which 8 were adults and the remainder juveniles. In two cases myxedema preceded the onset of diabetes. It is of interest that in the juvenile patients the presence of myxedema did not seem to affect the severity of the diabetes, the insulin requirement, or the danger of ketosis.

INTERRELATIONS OF DIABETES AND GASTROINTESTINAL DISEASES

Effect of Gastrointestinal Disturbances on Diabetes

Acute gastroenteritis, either infectious or of toxic origin (food poisoning) should always alert the physician and the diabetic patient alike to the imminent threat of ketoacidosis and coma. This is especially true in the more severe unstable type of diabetes in children and adults requiring exogenous insulin for control and also in milder stable diabetes controlled by oral hypoglycemic agents alone. The accompanying symptoms of nausea, vomiting, anorexia, abdominal pain, and diarrhea not only interfere with absorption of food and liquids but also deplete the

body of water and electrolytes. Fever, starvation, and dehydration aggravate diabetes and increase the requirement for insulin. Thus, a vicious circle is established. If the usual dose of insulin is taken without food, severe hypoglycemia may develop; while if insulin is omitted, the ensuing hyperglycemia and glycosuria leads to further loss of water and electrolytes via the urinary tract. Accordingly, food and insulin have to be titrated to avoid hypoglycemia on one hand and ketosis on the other. The usual practice is to reduce the daily insulin requirement to two thirds or half the dose and supplement this with small doses of regular insulin dependent upon the results of frequent analysis of the urine for glucose and acetone. Small amounts of easily digested bland foods and sugar-containing drinks may be given as tolerated.

Effect of Diabetes on the Gastrointestinal Tract

Diabetes may affect the gastrointestinal tract in several ways. Anorexia, nausea, vomiting, and abdominal pain are characteristic symptoms of uncontrolled diabetes with ketoacidosis and coma. The gastrointestinal symptoms may be related in some unknown fashion to the toxic effects on the gastrointestinal tract of excessive ketone body accumulation, but this remains to be established. The abdomen in diabetic acidosis may simulate an acute surgical abdomen, e.g., acute appendicitis, in every respect, even to the elevated white blood cell count.

Occasionally, unexplained diarrhea, occurring chiefly at night has been encountered in patients with diabetes (56). The cause has not been established, but it is generally believed that "diabetic nocturnal diarrhea" probably represents an unusual manifestation of diabetic neuropathy involving the sympathetic innervation of the large bowel (44). Previous history of poor diabetic control and diabetic peripheral neuritis has been reported to precede the diarrhea in a large percentage of cases. Usually there is a definite elevation of spinal fluid protein. No abnormalities have been noted on proctoscopy or radiographic examination of the large bowel. The Joslin group has reported good therapeutic results with the use of crude liver extract, starting with the injection of 2 to 4 ml. of the extract daily for a few days followed by maintenance doses of 1 to 2 ml. weekly (31). However, the efficacy of this or any mode of therapy is difficult to evaluate because the syndrome tends to run a chronic intermittent course marked by remissions and relapses of variable duration.

The author (29) has encountered several diabetic patients with recurrent episodes of unexplained nausea and vomiting lasting for several days. All of the patients have been younger individuals with long-

standing diabetes complicated by advanced retinopathy and nephropathy. Radiographic examinations of the upper gastrointestinal tract have revealed gastric atony and delay in emptying time of the stomach but no intrinsic lesions. One patient was thought to have pyloric obstruction possibly secondary to an old ulcer, by radiographic studies. Several weeks later the patient died of renal disease, and at necropsy the stomach, pylorus, and duodenum were found to be normal. The roentgenograms of the small bowel have revealed patterns in some of these patients characterized by aggregates of barium referred to as "puddling" or as the "moulage" sign, resembling those seen in nutritional disturbances such as sprue and similar deficiency diseases (12). Apparently, these radiographic abnormalities can also occur without clinical symptoms. Crozier, *et al.* observed the above-mentioned radiographic abnormalities in the small bowel without clinical symptoms in 7 of 82 children with diabetes examined at random.

Although all the diabetic patients with the syndrome of nausea and vomiting had advanced renal disease, they were not in uremia. In most cases diabetic peripheral neuropathy was either present or had preceded the syndrome. The episodes were not correlated with the degree of control of the diabetes, although in most cases the diabetes was markedly unstable. It is suggested that the syndrome may be related to diabetic involvement of the autonomic nerves in the upper gastrointestinal tract in a fashion similar to the alleged involvement of the lower tract. In the author's experience, development of the syndrome is a rather serious omen.

INTERRELATIONS OF DIABETES AND DISEASES OF THE CEREBRUM

Cerebral Regulation of Carbohydrate Metabolism

The central nervous system exerts a dual control on blood glucose homeostasis via the smoothly integrated activities of the two divisions of the autonomic nervous system. The sympathetic branch counteracts a fall in blood sugar by stimulating the secretion of adrenalin from the adrenal medulla. This system is termed the *sympatho-adrenaline apparatus.* The parasympathetic branch checks the rise in blood glucose by stimulating the release of insulin by the pancreas, and this together with the pancreas constitutes the *parasympatho-insulin apparatus* (23). In 1858 Claude Bernard produced the first evidence for the existence of sympathetic nerve centers in the brain capable of influencing carbohydrate metabolism. By puncture of the floor of the fourth ventricle, an operation termed *piqûre diabètique,* he was able to produce glycosuria.

Since then, interconnected suprabulbar and medullary centers that affect blood sugar level have been demonstrated for both branches of the autonomic division of the nervous system (23). In all probability the autonomic system has centers in each phylogenetic layer of the brain.

In response to a falling blood glucose concentration, impulses pass through the hypothalamic, pontine, and bulbar centers down to the lateral horn cells of the spinal cord, the lateral ganglia, and finally through the splanchnic nerves to the liver and adrenal medullae (38). *Splanchnic impulses to the liver accelerate glycogenolysis directly* while the impulses to the adrenal medulla stimulate release of epinephrine to the blood stream which, in turn, further enhances glycogen breakdown in the liver. As a result, greater amounts of glucose are liberated into the blood stream and the level of glucose rises. In response to a rising level of blood glucose, parasympathetic centers situated in the hypothalamus and the bulb are stimulated. Impulses arising from these centers pass to the right vagus nerve and on through the vagus to the islets of Langerhans (8). As a result, insulin output increases and blood glucose falls. Thus, both divisions are harmoniously balanced to maintain the blood glucose level constant within narrow physiological limits. Although the end organs involved, that is, liver and pancreas, can respond directly to gross changes in blood glucose levels without the intervention of autonomic reflex stimulation, the latter may permit them to respond to slight changes in glucose levels and thus permit a finer control.

Effect of Cerebral Lesions on Carbohydrate Metabolism

Clinically, disturbances in carbohydrate metabolism simulating diabetes mellitus may be associated with certain types of cerebral lesions. Hyperglycemia and glycosuria have been observed on occasion in cases of hemorrhage into the subarachnoid space and/or the brain tissue at the base of the brain following cerebral trauma, cerebrovascular accident, or ruptured aneurysm of one of the cerebral arteries (31, 60, 63). Space-occupying lesions, such as tumors or cysts at the base of the brain in the region of the hypothalamus, are also apt to be associated with hyperglycemia and glycosuria (60). The exact mechanisms involved in the hyperglycemia and glycosuria are not completely understood but are thought to be related, at least in part, to degenerative changes in the sympathetic centers or conducting fibers that are due to injury or pressure (23). This results in hyperexcitation of the sympathetic division of the autonomic nervous system and therefore in accelerated glycogenolysis. In view of the presence of parasympathetic as well as sympathetic centers in the areas of the brain concerned, it is not clear at present

why associated disturbances in carbohydrate metabolism are more apt to be manifested by hyperglycemia and glycosuria rather than by hypoglycemia. Apparently, disturbances in local metabolism or function enhance sympathetic control. In the hypothalamus the sympathetic centers are normally predominant (23). *Hyperglycemia and glycosuria can be* produced experimentally by injury to the hypothalamus (53) in animals, as well as by the effect of a tetanizing electrical (22) current on the hypothalamus. The effect of the latter seems to be peculiar to the hypothalamus because stimulation of other parts of the brain, e.g., the *cerebellum, does not produce glycosuria* (22).

The disturbances in carbohydrate metabolism associated with any of the cerebral lesions discussed above may be difficult to distinguish from those of diabetes mellitus, and can be severe enough to require exogenous insulin for regulation. In cases of vascular lesions, the disturbances usually are transitory and tend to clear rapidly with stabilization and/or recovery. In patients with pre-existing diabetes, cerebrovascular accident with hemorrhage may markedly aggravate the diabetes. This is well illustrated by the following case.

In 1956, a 65-year-old known diabetic was admitted to the hospital in deep coma. Examination revealed bilateral complete paralysis of upper and lower limbs. The spinal fluid was grossly bloody. The urine showed 4+ sugar and 4+ acetone. The history obtained from the wife revealed that diabetes had been present for several years but was mild enough to be successfully treated by dietary restriction alone. In addition to intravenous glucose infusions and later tube feedings, the administration of 80 to 100 units of regular insulin daily in divided doses was necessary to control the diabetes and prevent ketosis. Gradually the insulin requirements decreased so that after several weeks control was possible by tube feedings alone. The patient has remained completely paralyzed and semicomatose up to the time of this writing.

Effects of Diabetes on Cerebral Metabolism and Function

Both severe insulin hypoglycemia and ketoacidosis in patients with diabetes mellitus result in deranged cerebral metabolism, mental confusion, and loss of consciousness. In both conditions oxygen consumption by the brain cells is reduced and cerebral metabolism depressed (23, 32). The reduced cerebral metabolic rate in hypoglycemic coma is due to an inadequate supply of glucose to the brain cells, whereas in diabetic acidosis and coma the depressed cerebral metabolism is related to the intracellular effects of excessive accumulation of ketone bodies, and probably to a lesser extent to the acidosis and to other factors yet to be defined (32). Studies by Kety have revealed a striking correla-

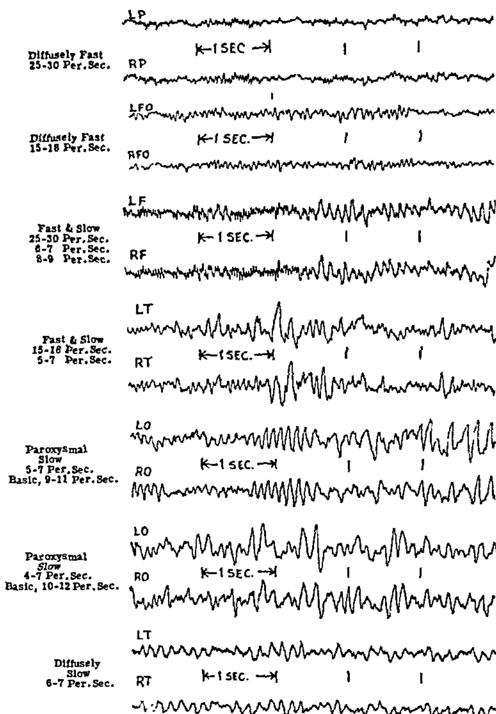


FIG. 45-6. Examples of the different types of abnormal EEG's in a series of 81 patients with diabetes mellitus (From Izzo, J L., Schuster, D. B., and Engel, G. L.: *Diabetes* 2 93, 1953.)

tion between mental state and cerebral metabolism. In conscious and alert normal individuals the mean value for oxygen consumption/100 gm. of brain/min. was found to be 3.3 ml. In patients who as a result of diabetic acidosis or hypoglycemia were *conscious but confused*, this value fell approximately 20 per cent to a mean value of 2.6 ml., whereas in comatose patients from any cause the mean value was only 2 0 ml./100 gm./min., a 40 per cent reduction from the normal figure. On the basis of studies on schizophrenic patients receiving insulin shock therapy, it has been estimated that the glycogen of the brain will support cerebral metabolism for about 90 minutes at the low level present in coma. This time period corresponds roughly to the time in which a patient may remain in deep coma before permanent damage is done to the brain. Protracted diabetic coma may at times result in death even though the chemical derangements are finally corrected, presumably because permanent damage to the brain has occurred.

Permanent abnormalities in the electroencephalogram have been reported to occur in 50 to 80 per cent of unstable diabetics with a history of frequent severe hypoglycemic reactions to insulin (17, 20). This represents a fivefold to tenfold increase over the 8 to 10 per cent figure for abnormal patterns found in the nondiabetic population (19). In the studies of Izzo, *et al.* (25), 60 per cent of the 81 diabetic patients studied had abnormal electroencephalograms. The different types of abnormal encephalograms encountered are illustrated in Figure 45-6. Of considerable interest is the finding that the incidence of abnormal records was significantly increased in patients with mild stable diabetes receiving no insulin as well as in those receiving insulin. Furthermore, although the patients receiving insulin showed a higher percentage of abnormal records, no difference was discernible between the stable and unstable patients on insulin therapy. The incidence and type of EEG patterns obtained in the three groups of patients discussed are shown in Figure 45-7. No significant correlation could be made between EEG type and blood sugar level, duration of diabetes, incidence of coma or acidosis, incidence of minimal dementia, or associated systemic or neurological disease. A greater incidence of slow waves was found, however, among patients who had frequent insulin reactions.

The basis for the cerebral dysrhythmia in diabetic patients is not clear at present. It may reflect subtle metabolic and functional disturbances of the brain cells, which are related in an unknown fashion to the general metabolic disturbances in diabetes. It has been suggested that abnormalities in the EEG may result from permanent damage to the brain cells as a result of severe protracted hypoglycemia owing to overtreatment of the diabetic state with insulin (20). While this may

be a contributing factor, the presence of identical abnormalities in patients without a history of severe hypoglycemia suggests that other factors are also concerned. Fabrykant and Pacella have suggested the possibility of a genetic or constitutional factor causing some of the EEG abnormalities in diabetes.

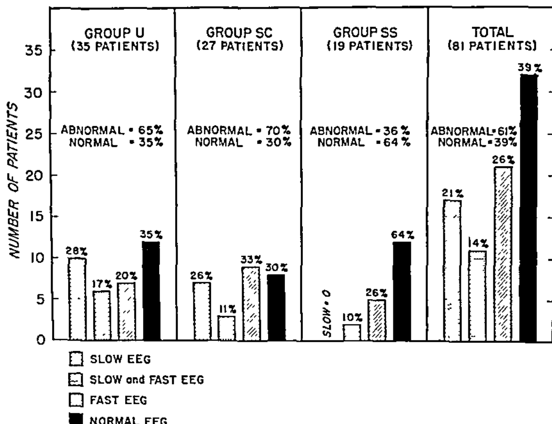


Fig. 45-7. Incidence and type of abnormal EEG's in a series of 81 patients with diabetes mellitus: Group U (the relatively unstable group), Group SC (the relatively stable group receiving insulin), and Group SS (the relatively stable group controlled by diet alone). (From Izzo, J. L., Schuster, D. B., and Engel, G. L.: *Diabetes* 2 93, 1953.)

Many of the abnormal records in diabetic patients closely resemble those seen in epileptics, but this hardly seems a sufficient basis for relating the two disorders or for providing a rationale for the use of anticonvulsive drugs, as others have suggested. Although anticonvulsive drugs have been claimed to be of value in the treatment of the unstable diabetic with a marked tendency toward frequent and severe hyperglycemia (17, 64), the actual value of these drugs in this condition has not been established, in the author's opinion.

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Chapter 46

LIPOATROPHIC DIABETES

James W. Craig and Max Miller

In 1951 R. D. Lawrence proposed the term lipoatrophic diabetes for a human disease syndrome with the following features: (1) generalized complete lipoatrophy involving both subcutaneous fat and other fat depots such as those within the abdomen, (2) diabetes mellitus with insulin resistance but with no tendency to develop ketosis even in the absence of administered insulin, (3) intense hyperlipemia with cutaneous xanthomata, (4) hepatomegaly, and (5) an elevated basal metabolic rate not explainable by hyperthyroidism.

Lawrence had described the clinical picture and autopsy findings of a patient with this syndrome in 1946 and had called attention to reports of two cases with most of these features which had been published by other authors. Since that time at least nine additional cases with generalized lipoatrophy and diabetes mellitus have been recognized in Europe and the United States.*

* Patients with diabetes mellitus and lipodystrophy whose loss of subcutaneous fat is not generalized but is usually limited to the upper half of the body will not be included in this description of lipoatrophic diabetes

CLINICAL AND LABORATORY FINDINGS

Some of the clinical and laboratory findings in the three cases fr Lawrence's 1946 report and the nine subsequently recognized patie with this syndrome are recorded in Table 46-1. Lipoatrophic diabe has occurred in both white and Negro patients and in both sexes. It l appeared at from 4 to 50 years of age, but in half the cases it has (veloped before the age of 16 years. Its occurrence in a pair of sist (Cases VI and VIII) is the only instance in which a family history the syndrome has been obtained. Because of the absence of su cutaneous fat in patients with this syndrome, the individual mus groups of the trunk and extremities and the superficial veins are read visualized as in an anatomic illustration and the females have a ratl masculine habitus (Fig. 46-1). Cutaneous xanthomata have occurred a minority of cases and have fluctuated in number and size, depend upon the degree of hyperlipemia. Hepatic enlargement has been scribed in all but one of these patients and splenomegaly has also be frequent. The diabetes has been of variable severity as measured the insulin requirement and the degree of hyperglycemia. The da maintenance dose of insulin was 200 or more units in five of the patier in other cases a glucose tolerance test was required to establish diagnosis of diabetes mellitus. Even in those patients with intense hyp glycemia and glycosuria, ketonuria has been absent, provided starvat or a stressful situation was not present. Under the latter circumstan the ketosis has not been severe or rapidly progressive and ketoacido has never been observed. For example, after 3 months without adm *istered insulin Case VI was excreting up to 200 gm. of glucose daily* l was free of ketonuria. The absence of ketosis is a particularly striki feature in the juvenile patients with this syndrome since, in this a group, diabetes is usually of the labile type and ketoacidosis occ promptly when insulin is withdrawn. Studies with C^{14}-glucose in Ca VI indicated that the rate of oxidation of glucose to CO_2 was similar that observed in normal subjects and in patients with stable diabet however, the rate of CO_2 production was abnormally high and the p centage of the expired CO_2 derived from glucose was less than norm These findings are indicative of an abnormally large CO_2 producti from nonglucose materials, possibly fat. Hennes and Shreeve adm istered 2-C^{14} acetate to Case XI and found an abnormally great a rapid incorporation of C^{14} into triglycerides and cholesterol with abnormally slow decline in this activity. Lawrence reported that insul like activity was found in the plasma of his patient by Bornstein's te

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TABLE 46-1. LIPOATROPHIC DIABETES

Case	Source of information*	Age at last report (years)	Race	Sex	Age at onset (years): Lipoatrophy	Age at onset (years): Diabetes mellitus	Maximum daily insulin dose (units)	Keto-nuria	Hyper-lipemia	Xan-thomata	Hepato-megaly	Maximum basal metabolic rate (Per cent)
I	Ziegler	38†		F	11	21	0	–	–	–	+	+57
II	McQuarrie	9†	W	M	3	4	284	–	+	–	+	normal
III	Lawrence	34†	W	F	26	26	2160	±	+	+	+	+177
IV	Hood	55	W	F	12 or less	41	24	±	+	–	+	+95
V	Aarseth	58	W	F	29 or less	50	68	–	+	–	+	+44
VI	Craig and Miller	21	N	F	<13	13	2000	±	+	+	+	+40
VII	Corner	22	W	F	<14	15	125	±	+	–	+	+45
VIII	Craig and Miller	17	N	F	<12	12	70	–	+	+	–	+15
IX	Conn	12	W	M	7	8	360	–	+	–	+	+27
X	Witzgall	41		F	10 or less	10	80	–	+	–	+	+64
XI	Schwartz	14	W	F	4 months	12	200	±	–	–	+	+20
XII	Conn	28	W	F	6	21	0	–	+	–	+	+7

* See References
† Died
W White
N Negro
M Male
F Female
\+ Present
– Absent
± Usually observed only in association with starvation, infection, or emotional upset.

nique but could not be quantitated because of interfering plasma lipemia. A high level of insulinlike activity was also detected in Case XI by the rat epididymal fat pad and diaphragm techniques as long as 5 months after the withdrawal of exogenous insulin. Hyperlipemia and hypercholesterolemia of sufficient degree to render the serum milky in

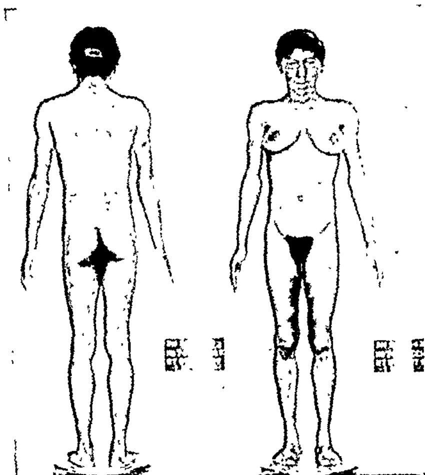


FIG. 40-1. Lipoatrophic diabetes (Case VI). The apparent preservation of mammary adipose tissue in the presence of generalized lipoatrophy is an interesting feature in this case.

appearance have been usual but not invariable findings; in some cases a reduction of the elevated blood glucose level has been accompanied by a decrease in lipemia. The basal metabolic rate has varied from extremely high levels in some cases to normal values in others, while the thyroidal I^{131} uptake and the level of serum protein-bound iodine have been normal when measured. In two cases thyroidectomy did not reduce

the basal metabolic rate to normal. No definite evidence of abnormal pituitary or adrenal function has been reported.

COURSE AND PROGNOSIS

In the majority of cases lipoatrophy has preceded the diagnosis of diabetes mellitus, sometimes by as long as several years; in a few cases the two conditions appeared to develop at about the same time. Three of the patients have died from 5 to 14 years after the development of the syndrome. One (Case II) died of gastrointestinal hemorrhage secondary to hepatic cirrhosis with portal hypertension; another (Case III) died of complications including infection, which followed a laparotomy for the removal of ovarian cysts; the cause of death in the third case (Case I) is unknown. In the eight patients who are currently alive, the syndrome has apparently existed from 1 to 13 years. Diabetes mellitus has not been of sufficient duration in these patients to determine whether or not the incidence of degenerative vascular complications will be the same as in the more common types of diabetes. Experience is also inadequate to predict the subsequent course of their chronic liver disease.

PATHOLOGIC FINDINGS

Knowledge of the pathologic changes in this syndrome has been gained from autopsies on two of the patients (Cases II and III) and from tissue biopsies in some of the others. Subcutaneous, intra-abdominal, and perinephric fat have been completely absent; the liver has shown increased or normal amounts of fat except for one case in which the hepatic content of neutral fat was reduced while the phospholipids and cholesterol were normal. The histologic changes in the liver have included fatty infiltration, portal cirrhosis, and, in one case, hypertrophic cirrhosis. In some cases the lymph nodes have been enlarged and have been described variously as showing dilated lymph sinuses filled with vacuolated eosinophilic material, chronic fibrosis, or inflammatory changes. The pancreas was said to be histologically normal in one case and to show slight chronic fibrosis in the other case in which it was examined; special stains for islet cell granules were not employed. The thyroid from one patient (Case III) showed many nodular areas of hyperplasia with small acini lined by high columnar epithelium and containing reduced amounts of colloid, while tissue obtained from another patient (Case I) by subtotal thyroidectomy was described as "colloid and foetal thyroid with extensive interacinar hyalinization." The other endocrine glands of the two autopsied patients appeared normal.

CAUSE AND PATHOGENESIS

The existence of at least 12 cases with many or all of the features of Lawrence's original case supports that author's belief that lipoatrophic diabetes represents a syndrome rather than a fortuitous coincidence of unrelated manifestations. Perhaps some of the patients with progressive cephalothoracic lipodystrophy and diabetes mellitus represent an incomplete form of the syndrome. If lipoatrophic diabetes is a syndrome, does it have a single cause that would account for its diverse manifestations? Although two cases have occurred in one family, the available data are inadequate for appraisal of the role of genetic factors in the development of this syndrome. The observation in animals that anterior pituitary factors can produce both diabetes mellitus and a mobilization of fat from adipose tissue to the liver suggests that dysfunction of this endocrine gland might cause lipoatrophic diabetes. However, there has been no evidence of abnormal anterior pituitary function in patients with this condition, and excesses of the well-characterized anterior pituitary hormones produce clinical syndromes that are distinct from lipoatrophic diabetes. Epinephrine is a hormone known to elevate both the blood glucose concentration and the basal metabolic rate and to promote the mobilization of fat from adipose tissue to the liver. Here again there is no good evidence for the presence of increased epinephrine in patients with lipoatrophic diabetes and a different clinical picture is associated with epinephrine-producing tumors. Although the nervous system can influence diverse metabolic functions including fat mobilization and carbohydrate metabolism, neurologic disease has been absent in most of these patients. Just as no single cause for this syndrome has been found, the pathogenesis and interrelationships of its various manifestations such as lipoatrophy, hyperlipemia, hyperglycemia, hepatic disease, and an elevated basal metabolic rate are obscure. Lawrence suggested that lipoatrophy was the primary defect in this condition; since fat could not be stored, it circulated in excess; and since there was little mobilizable fat, ketosis did not occur. He attributed the hyperglycemia and insulin resistance to the impossibility of converting blood glucose into stored fat in the absence of normal fat depots. A cause-and-effect relationship between the loss of depot fat and the abnormality of carbohydrate metabolism is also suggested by the relatively high prevalence of defects in glucose metabolism in patients with progressive cephalothoracic lipodystrophy whose loss of subcutaneous fat is limited to the upper half of the body. However, factors other than a loss of fat depots must be involved, since generalized lipoatrophy has been observed without diabetes mellitus. From these

speculations it can be seen that the cause of lipoatrophic diabetes is unknown, its pathogenesis is not proved, and it remains, in the words of Lawrence, a "fascinating, rare, and obscure syndrome."

THERAPY

Since the cause of this syndrome and the pathogenesis of its various manifestations are unknown, management of the diabetes is the only effective treatment available. The principles of diabetic therapy are the same as those employed in patients with the more common form of stable diabetes. Lowering the dietary fat intake does not appear to decrease the lipemia, nor has a high fat intake been demonstrated to restore depot fat. Accordingly, there is no indication for a drastic alteration in dietary fat. As described above, some of the patients require large doses of insulin. Experience with tolbutamide in patients with this syndrome is limited; the drug reduced the insulin requirement moderately in at least one case but was entirely ineffective in another patient. Successful diabetic control has been associated with a decrease in hyperlipemia and a regression of cutaneous xanthomata.

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Chapter 47

DIABETES ASSOCIATED WITH ACROMEGALY, HYPERADRENOCORTICISM, HEMOCHROMATOSIS, PANCREATITIS, PANCREATECTOMY, AND CANCER

Max Miller

ACROMEGALY

That the hormonal function of the pituitary gland is in some way related to carbohydrate metabolism is evident clinically from the increased frequency of diabetes in patients with acromegaly. Collected series from the literature (Table 47-1) totaling almost 500 cases reveal an incidence of diabetes of 25 per cent. In some series additional numbers of cases with mild glycosuria and impaired glucose tolerance tests perhaps will swell this number up to 50 per cent of all acromegalics, but detailed data on this point are usually not available. The disturbance in carbohydrate metabolism is probably related to the diabetogenic effect of growth hormone (Chapter 17) which is elaborated in excess amount in acromegaly, and to the concomitant secretion of corticotropin in the very rare case of coexisting acromegaly and Cushing's syndrome. Exhaustion of the islet cells of the pancreas from stimulation by growth hormone also may be an important factor.

The diabetes, when present, is usually mild and of the stable type,

occasionally insulin-resistant, requiring more than 100 units of insulin daily. Since acromegaly occurs predominantly in adult life, beginning most frequently in the third decade, and the diagnosis of diabetes is made subsequently from 1 to 22 years later, one would not expect many cases of the labile type of diabetes to occur. In 3 of 16 patients in Coggeshall's series deaths were due to diabetic coma, but 2 of these were in the preinsulin era. It is consequently difficult, if not impossible, to characterize the diabetes as being "pituitary" in type, and one may say that the clinical features are similar to those seen in patients in any diabetic clinic. The severity of the diabetes, as measured by insulin requirements, often waxes and wanes with activity of the acromegalic

TABLE 47-1. INCIDENCE OF DIABETES MELLITUS IN ACROMEGALY

Author	*Number of cases with acromegaly*	*Number of cases with diabetes*	*Percentage of acromegaly with diabetes*	*Reference*
Hansemann, D.	97	12	12.4	*Berl. Klin. Wchnschr.* *34*:417, 1897.
Borchardt, L.	156	60	38.0	*Deutsch. med. Wchnschr.* *34*:946, 1908.
Coggeshall, C., and Root, H. F.	153	26	17.0	*Endocrinol.* *26*:1, 1940.
Shepardson, H. C.	15	6	40.0	*J. Nerv. & Mental Dis.* *99*:862, 1944.
McCullagh, E. P.	76	21	36.2	*Diabetes* *5*:223, 1956.
Total	497	125	25.2	

state. When the lesion of the pituitary becomes inactive, either spontaneously or as a result of treatment, the diabetes may become milder or may, for all practical purposes, disappear.

With the improvement in treatment of acromegaly with irradiation to the pituitary, use of hormones to inhibit adenohypophyseal activity, improved handling of the secondary manifestations such as hyperthyroidism, diabetes mellitus, and hypopituitarism, and better control of the secondary complications such as infection and heart failure, life expectancy has been significantly increased. Thus, sufficient time is present in some cases to permit the development of some of the usual complications seen in chronic diabetes. McCullagh reports 3 cases of typical diabetic retinopathy in 21 acromegalic diabetic patients. The duration of diabetes in these 3 cases was 8, 2, and 7 years, respectively. In these 3 cases no mention was made of renal involvement seen often

in long-term diabetes, and it is surprising that only a very few cases of this sort have been noted in the literature.

Treatment with diet and insulin of the diabetes seen in acromegaly is the same as in nonacromegalic patients with diabetes. Oral hypoglycemic agents of both the sulfonylurea and biguanide types are effective in the milder, stable type diabetics. As indicated above, amelioration of the acromegalic state, which may occur spontaneously or as a result of therapy by irradiation of the pituitary or with large doses of estrogenic hormones, in many cases brings about a striking improvement in the diabetes. Of interest is the report of McCullagh in 5 acromegalic women with mild diabetes in whom he used from 1.0 to 5.0 mg. per day of ethinyl estradiol. Over a period of months, coinciding with recession of acromegalic symptoms and signs, improvement was consistent in all 5 patients, with the glucose tolerance test becoming normal or near normal during therapy.

HYPERADRENOCORTICISM

More than a quarter of a century has elapsed since Harvey Cushing first described the clinical syndrome of obesity, hypertension, virilism in females, diabetes, plethora, and osteoporosis. Although at first it was claimed to be caused by hyperfunction of the pituitary basophil cells, in recent years most workers in the field have agreed that the clinical picture presented is that of hyperadrenalism, owing to the effects of a prolonged excessive production by the adrenal cortex of 11,17-oxygenated corticoids. The similarity between this syndrome and the hyperadrenal state induced by the administration of corticotropin or cortisone, and the more consistent finding pathologically of adrenal cortical adenoma, carcinoma, or bilateral hyperplasia have been the prime reasons for this change in concept of pathogenesis.

Impaired carbohydrate tolerance is almost a characteristic part of the syndrome, being present in more than four fifths of all cases (Table 47-2). In 174 collected cases, "frank" diabetes (presumably cases with fasting hyperglycemia and those requiring insulin for control) was found in 26 per cent when adequate data on this point were available, and an additional 55 per cent had impaired glucose tolerance tests. Clinically the diabetes is stable in type, usually mild, relatively insensitive to insulin, and associated with a negative nitrogen balance not completely correctable by insulin administration. Ketosis is almost always absent or, if present, usually mild. In McCullagh's series the highest dose of insulin required was 90 units per day.

An interesting difference in intermediary metabolism in patients with

Cushing's syndrome has been described in recent years. In normal individuals the administration of glucose produces a rise in blood pyruvate and lactate. In diabetic subjects these metabolites either fail to rise or the rise is delayed. In contrast, in some cases of Cushing's syndrome the fasting levels of pyruvate and lactate are elevated and, following a glucose load, the diabetic glucose curve is accompanied by high levels of pyruvate and lactate. Unfortunately, the possible usefulness of this test has not been reported as yet in mild or borderline cases.

TABLE 47-2. INCIDENCE OF DIABETES MELLITUS IN CUSHING'S SYNDROME

Author	*Cushing's Syndrome Number of cases*	*Normal glucose tolerance test Number of cases*	*Impaired glucose tolerance test Number of cases*	*"Frank diabetes" Number of cases*
Sprague	67	10	35	22
Plotz	33	2	26	5
Soffer	40	8	21	11
McCullagh	34	13	14	7
Total	174	33 (19%)	96 (55%)	45 (26%)

Since vascular disease including hypertension is so commonly seen in Cushing's syndrome, it might be anticipated that there would be considerable difficulty in defining the role of diabetes in its development. Until relatively recently the long-term prognosis usually has been short, Plotz, *et al.*, reporting half of the patients dying within five years. Consequently, few cases survive long enough for the typical diabetic vascular complications to become manifest. Skillern and McCullagh, however, have reported two cases with typical diabetic retinopathy in a total of 34 cases. One had the features of Cushing's syndrome for 11 years and diabetes for 9 years before the retinopathy was found, while in the other the retinopathy followed 8 years of diabetes. At the Mayo Clinic, Sprague and Hoffman (personal communication) have found, among 135 cases, 5 cases of "fairly characteristic" fundal changes, including capillary aneurysms and so-called punctate hemorrhages. Three of the five had rather severe diabetes, a fourth had two capillary microaneurysms, with a normal fasting blood sugar and an abnormal glucose tolerance test, while the fifth patient had a typical diabetic retinopathy with a *normal* glucose tolerance test. There is not a single recorded instance of intercapillary glomerulosclerosis in cases of Cushing's syndrome, but it must be noted that studies specifically directed toward

this point have not been undertaken. At any rate, from the above data it seems safe to conclude that diabetes of nonpancreatic origin, if allowed to proceed long enough, will be associated with vascular degenerative lesions in the eye. Some other factor or factors besides insulin lack per se must be responsible for the dread complications of diabetes.

Table 47-3 shows the type of adrenal pathology in 130 cases found

TABLE 47-3. ADRENAL PATHOLOGY IN CUSHING'S SYNDROME

Author	*Number of cases*	*Carcinoma*	*Benign adenoma*	*Hyperplasia*
Plotz	97	16	11	58
Soffer	33	10	8	5
Total	130	26 (20%)	19 (15%)	63 (48%)

either at post-mortem examination or at operation. Complete cure is most likely to occur if a benign cortical adenoma can be removed, but this represents only a small fraction of the total. Bilateral adrenalectomy for adrenal hyperplasia is the treatment of choice for this group, provided adequate post-operative and maintenance therapy with hydrocortisone and cortisone is given. Before these agents were available, more than half the patients with Cushing's syndrome died after surgery. Even in those patients with adrenal cortical carcinoma, significant remissions can be obtained, provided recognition and treatment are not unduly delayed (Soffer). In those cases where no adrenal pathology is demonstrable at operation irradiation of the pituitary alone, or combined with unilateral adrenalectomy (Soffer), will produce improvement in many instances.

The diabetes will improve in almost all cases and will disappear completely in more than 50 per cent of patients where surgical treatment or irradiation has been successful. The improvement in carbohydrate tolerance may be slow in some instances, and even when normal glucose tolerances are obtained some impairment of reserve may still be present. The administration of small amounts of cortisone (10 to 15 mg.) may provoke glycosuria under these circumstances.

IATROGENIC HYPERCORTICOIDISM

In contrast to the high incidence of diabetes found in Cushing's syndrome, diabetes develops in less than 1 per cent of patients given

continued ACTH or cortisone for therapeutic reasons (Bookman). In most cases reported of this sort, either a family history of diabetes can be elicited or a story of glycosuria prior to treatment. The diabetes is usually transient, disappearing when steroid administration is stopped. Steroid diabetes induced by ACTH or corticoids, either in animals or man, has these characteristic features: (1) insensitiveness to insulin, (2) striking diminution of glycosuria with fasting in the absence of insulin, and (3) negative nitrogen balance owing to the catabolic effects of these hormones even in mild degrees of this form of diabetes. In one of the 5 cases reported by Bookman, insulin requirements ranged as high as 110 units daily.

The problem of using steroids and corticotrophin in patients with a known diabetic family history or established diabetes must be considered on an individual basis. If one of these agents represents the drug of choice and the only one known to control the complicating basic disease, then the possibility of precipitating diabetes or worsening the existing diabetic state should be ignored, provided that adequate insulin can be given. In certain instances, insulin requirements may actually increase slightly or not at all, and it has even been reported that some cases of insulin-resistant diabetes improve with steroid therapy, presumably by altering the rate of production or disappearance of insulin antibodies. In the management of the induced diabetic state it must be kept in mind that the steroids also may cause reduction in the renal reabsorption of glucose. Emphasis on blood sugar as well as urine sugar tests consequently becomes a necessity.

HEMOCHROMATOSIS (BRONZE DIABETES)

Hemochromatosis is a rare disorder of metabolism characterized by the deposition throughout the body of abnormally large amounts of hemosiderin, an iron-containing pigment. In the liver and pancreas, hepatomegaly with cirrhosis and diabetes mellitus, respectively, are the consequences, fibrosis being an important factor; in the skin abnormal pigmentation is found, varying from blue-gray to bronze in color, from the presence of iron pigment and increased amounts of melanin; loss of hair, testicular atrophy, and impotence are often seen, probably secondary to liver dysfunction, with impairment of estrogen inactivation; and, finally, arrhythmias and congestive heart failure may result from the cardiac deposits of hemosiderin. The disease occurs predominantly in males, as indicated by a 13:1 ratio of males to females in 513 cases collected from the literature. The majority of cases are diagnosed in later life, usually between the ages of 40 and 60 (68 per

cent), but the three cardinal signs of the disease, pigmentation, hepatomegaly, and diabetes seldom appear simultaneously, but rather in the order listed above.

The accumulation of Fe in hemochromatosis is slow and progressive, taking place over many years. Normally, about 4 to 5 gm. of Fe are present in the body of an adult, mostly in hemoglobin (70 per cent); a variable amount, about 15 per cent (0.4 to 0.8 gm. Fe), is contained in the storage Fe protein, ferritin, and only a slight amount in the form of hemosiderin granules. In hemochromatosis the body may contain some 25 to 50 gm. of excess Fe as hemosiderin, which microscopically appear as visible brownish granules containing as much as 35 per cent Fe by weight. It has been postulated that hemosiderin represents ferritin material in which the iron hydroxide micelles may have enlarged abnormally to form cross links between micelles of adjacent molecules. Since Fe excretion in man is normally negligible (1 mg. Fe or less per day) it is clear that absorption rather than excretion controls the amount of body iron. In the normal subject a mucosal block rejects more than 90 per cent of the 10 to 20 mg. Fe in the usual nutritionally well-balanced diet, but in hemochromatosis the rate of Fe absorption is much increased, as indicated by radioactive iron studies (20 per cent and 45 per cent compared with 1.5 per cent to 4.4 per cent in controls). If Fe then were absorbed at a rate of 4 to 6 mg./day instead of 1 mg., it would take 20 years to accumulate 20 to 30 gm. in the body. The rarity of this disease in the female has been explained by her ability to rid herself of the absorbed iron by menstruation and pregnancy. Most of the cases in females, consequently, have been diagnosed after the age of 45. In Sheldon's series only 1 of 13 women (8 per cent) was diagnosed as having hemochromatosis before the age of 45, in contrast to 108 of 279 men (39 per cent).

In general, the diabetes of hemochromatosis is usually the last manifestation of the disease to appear. In the early stages the diabetes can be mild and easily controlled by diet alone. In those cases taking insulin, the requirements do not appear to be markedly different from those of any group of diabetic patients, and the majority present no problem in control. In some instances, however, insulin resistance has been reported. In one series, 6 of 47 cases required more than 100 units daily, one of whom died in diabetic coma despite being given 1,600 units a day in the last 3 days of life. Some patients in the late stages show marked instability of blood sugar with a tendency to ketoacidosis and hypoglycemia, characteristics similar to those seen in juvenile diabetic subjects or in completely depancreatized patients. This may be related to absolute insulin deficiency caused by complete destruction of the islets

from the deposition of hemosiderin and the resultant fibrosis. It is interesting in this regard to note that all reported cases of hemochromatosis treated with sulfonylurea compounds have failed to show any hypoglycemic response.

Prognosis and Complications

Sheldon, in 1935, reported the average duration of life in 89 instances of hemochromatosis from the time medical advice was first sought to the time of death to be 18½ months. Diabetic coma was the cause of death in 50 per cent, cirrhosis of the liver in 11 per cent, infection in 18 per cent, and carcinoma of the liver in 7 per cent. Myocardial failure from pigment fibrosis in the heart was the causative factor in a few of the remaining cases. With the modern treatment of diabetes with insulin, duration of life has been significantly increased. Boulin (1944) found, in 70 cases, the average duration from the onset of diabetes to be 4 years, with extremes from 1 to 20 years. Of 27 fatal cases reported from the Joslin Clinic in 1951, the average duration was 4.9 years.

With this relatively short average duration of diabetes, even in recent times, it is not surprising that the degenerative vascular complications of diabetes have not been conspicuous. Lonergan and Robbins (1959), reviewing 62 fatal cases of hemochromatosis from various Boston hospitals, found not a single case of intercapillary glomerulosclerosis. Although the average duration of the diabetes was only about 4.3 years, 21 of the 62 cases had had diabetes for 5 years or longer, and 8 had a duration of 10 years or more. Comparing this with other studies of Robbins they concluded that the diabetes associated with hemochromatosis did not predispose to renal vascular disease and that insulin deficiency per se, therefore, was not the factor necessary for its development. Unfortunately, not enough data on retinal changes were available in this series. On the other hand, Duncan, *et al.*, found both intercapillary glomerulosclerosis and diabetic retinopathy in a patient with pancreatic diabetes owing to chronic pancreatitis with fibrosis and calcification. From this case Duncan came to the opposite conclusion, that the degenerative complications of diabetes were the consequences of the insulin deficiency. Duncan also cited 3 cases of hemochromatosis with diabetic retinopathy; one of these had diabetes of 15 years' duration, a second had long-standing diabetes, but no duration was given in the third case. Additional careful studies of the retina and kidneys for vascular complications in patients with hemochromatosis and diabetes of long duration must be made, since this is an issue of great importance in providing a possible clue to the pathogenesis of the complications of diabetes.

Diagnosis and Treatment

Hemochromatosis should be considered in patients presenting the combination of skin pigmentation, cirrhosis, and diabetes. Cardiac failure, loss of hair, testicular atrophy, and impotence, when the triad is present, add further support to the diagnosis. Hypertension is strikingly rare. Laboratory confirmation depends upon the demonstration of excessive iron stores in the form of hemosiderin pigment by biopsy of the skin or liver, and by examination of the urinary sediment or sternal marrow. Where the determinations are available, the findings of an elevated serum iron and saturation of the iron-binding capacity of the serum provide a simple and accurate test for proving the presence of an excess of iron.

Meticulous control of the diabetes primarily to avoid ketoacidosis and hypoglycemia is essential to prolong life. In recent years the removal of the excessive iron stores by the simple expedient of weekly or biweekly phlebotomies of 500 ml. of blood has been reported by Davis (1953) to produce significant clinical improvement. Each venesection represents the removal of 200 to 250 mg. of iron from the blood, which is replaced by an equivalent amount of iron from tissue stores, as indicated by the fact that patients with hemochromatosis will maintain their hematocrit at normal levels on this regimen. In about two or three years, therefore, most of the excess iron could be removed. The reversibility of the process will be dependent on the amount of tissue damage (fibrosis) present at the time of initiating the phlebotomies and to what extent the iron per se is responsible for the tissue damage. So far, evaluation of the long-term effect of this apparently logical method of treatment on the course of the disease has not been reported.

PANCREATITIS

Pancreatitis can be defined as the inflammation resulting from escape of the pancreatic secretion into the parenchyma of the gland. From a clinical standpoint it can be classified as acute and chronic. The two forms are essentially the same disease, the separation clinically between them being arbitrary, based on the duration of disease (usually more than 3 months) or the number of attacks. The findings of pancreatic calcification supports the diagnosis of chronic pancreatitis.

Acute Pancreatitis

alcohol. Cholelithiasis occurs two to four times more frequently in patients with pancreatitis than in the general population. Prostration and shock are associated with the more severe forms of the disease when hemorrhage and necrosis are taking place. The most significant laboratory test in establishing the diagnosis is the determination of serum amylase, which rises early in the disease and may return to normal levels within 24 to 72 hours. The mortality in 569 cases collected from various series was 16 per cent.

The occurrence of glycosuria and hyperglycemia in these patients with acute pancreatitis has not been systematically determined. From incomplete studies the incidence of glycosuria is at least 11 per cent. The more severe the disease the greater the percentage of positive tests, reaching 35 per cent in fatal cases. Where blood sugars have been reported, hyperglycemia is found with more regularity, in from one fourth to one half of severe cases. When glucose tolerance tests are performed, an impairment has been noted in almost every instance. The diabetes so diagnosed is usually transient, disappearing with the subsidence of the acute process, and in only 2 per cent of 2,855 collected cases did the diabetes become permanent.

Chronic Pancreatitis

Recurrent attacks of acute pancreatitis, the finding of pancreatic calcification on x-ray, or confirmation by biopsy at surgical exploration determine the diagnosis of chronic pancreatitis. With progressive destruction of the pancreas both exocrine and endocrine function become impaired. Eventually, advanced malnutrition, greasy and bulky stools and diabetes often become established features of the disease.

TABLE 47-4. INCIDENCE OF DIABETES MELLITUS IN CHRONIC PANCREATITIS

	Number of cases	*Diabetes Number of cases*	*Per cent with diabetes*
Without stones	322	41	13
Stones present	263	119	45

Since pancreatic calcification is usually a late manifestation of chronic recurrent pancreatitis and is associated with a greater destruction of pancreatic tissue a much higher incidence of diabetes and steatorrhea occurs when this finding is present. Table 47-4 shows the incidence of diabetes in chronic pancreatitis with and without stones.

Because of the relative infrequency of pancreatitis (1 case in every 400 necropsies) it would not be expected to be a causative factor in any significant fraction of the diabetic patients seen in practice. Sprague reported that only 24 cases of diabetes associated with chronic relapsing pancreatitis were seen in the years 1939 to 1945 and that these represented 0.3 per cent of all the cases of diabetes seen at the Mayo Clinic during that period.

Characteristics of Diabetes Associated with Chronic Pancreatitis

In most cases the diabetes becomes apparent only after several bouts of the pancreatitis over a period of several years. In the beginning the diabetes may be transient or mild, becoming permanent and increasingly severe as the destruction of the pancreas increases with each successive attack. The diabetes tends to be labile in character in the severe cases, as would be anticipated where complete insulin deficiency is present. The diabetes will also fluctuate during acute flare-ups of the pancreatitis, as would occur in any case of diabetes during a painful or febrile illness. During periods of remission insulin requirements seldom exceed 30 or 40 units daily. In Sprague's series, one third of the cases did not require insulin. With terminal malnutrition usually associated with steatorrhea, insulin dosage frequently can be reduced.

Diabetes resulting from destruction of the islet cells from chronic pancreatitis has been stated by some authors to be characteristically free of vascular complications. However, there are at least two documented cases of diabetic retinopathy and intercapillary glomerulosclerosis following recurrent chronic pancreatitis in the literature. In one case (Sprague) the patient developed relapsing pancreatitis at the age of 27 and became diabetic 8 years later. After a further 9 years he was found to have a specific diabetic retinopathy and probably intercapillary glomerulosclerosis. In the second case (Duncan) the patient had symptoms of steatorrhea for 21 years with extensive calcifications of the pancreas by x-ray. Diabetic retinopathy was diagnosed clinically and intercapillary glomerulosclerosis was found at autopsy. Diabetes had been present for 29 years but there was no family history of diabetes.

Acute Pancreatitis Complicating Diabetes

Acute pancreatitis can occur as a complication of existing diabetes mellitus. Its presence can frequently go unrecognized because of the intensification of the diabetes. Acidosis and coma may develop with great rapidity because of the involvement of the pancreas. The abdominal symptoms and signs of pancreatitis are almost always impossible to differentiate from those seen in diabetic acidosis. When this compli-

cation occurs the prognosis is grave, the majority of cases going on to death. Most of the reported cases have been undiagnosed during life, but shock, resistance to diabetic therapy, and the finding of a high serum amylase should suggest the diagnosis.

PANCREATECTOMY

The surgical removal of part or all of the pancreas in man for therapeutic reasons in recent years has greatly extended our knowledge of pancreatic diabetes. Pancreatectomy has been performed for carcinoma of the pancreas, hyperinsulinism, and for chronic recurrent pancreatitis. At least 90 to 95 per cent of the pancreas must be removed before clinical diabetes develops. As long as some functioning islet cells remain, the diabetes is usually stable and relatively mild, requiring only small amounts of insulin. After total pancreatectomy, however, the diabetes is characteristically extremely labile and difficult to control. Ketoacidosis and hypoglycemic shock are frequently cited as causes of death in patients who have undergone successful total pancreatectomy for their underlying disease. Because of these hazards many clinics have abandoned this method of surgical therapy.

Insulin requirements in patients with complete ablation of the pancreas usually do not exceed 50 units daily. In 10 cases reported by McCullagh (1958) the average insulin dose was approximately 25 units per day with a range from 10 to 50 units.

Vascular Complications

There seems to be no doubt that vascular complications will develop in some cases of diabetes of purely pancreatic origin. These have been described in hemochromatosis and in chronic pancreatitis with diabetes, and total pancreatectomy is no exception to the rule. Burton describes a 60-year-old woman who had a total pancreatectomy for hyperinsulinism. There was no family history of diabetes. Diabetes developed postoperatively and was controlled on 40 units of insulin daily. Three years later diabetic retinopathy was diagnosed and follow-up examinations 6 years after revealed even more retinal involvement. Urinary findings were not mentioned.

CANCER AND DIABETES

A relationship between cancer and diabetes has long been discussed but has been difficult to establish on sound statistical grounds. A recent long-term study by Joslin (1959) of 1,026 adult diabetic patients observed from the onset of diabetes to death, or to a 15-year follow-up

point if alive, showed no significant difference in the number of cases of cancer from the expected value in nondiabetic subjects. Although diabetes may not predispose to cancer, if the above results are valid, there is some evidence to suggest that altered carbohydrate metabolism as well as overt diabetes appears to be significantly associated with neoplasia. Glicksman performed standard glucose tolerance tests on 950 consecutive unselected patients with tissue diagnosis of cancer or of a particular benign lesion. A test was considered positive only if all three of the following criteria were present:

1. The true blood sugar level rose to more than 200 mg. per 100 ml. at any one time during the test.

2. The blood sugar level was more than 100 mg. per 100 ml. at the two-hour point.

3. The blood sugar level did not return to the fasting level in three hours.

The results in the two groups are recorded in Table 47-5. All tests

TABLE 47-5. INCIDENCE OF DIABETES IN CANCER PATIENTS

	Number	*Per cent diabetic*
All cancer patients	628	36 7
All "benign" patients	322	9 3

were performed in the hospital under standard dietary conditions (200 to 250 gm. of carbohydrate).

Of 158 patients with cancer of the endocrine organs, 45 6 per cent had diabetic glucose tolerance curves, compared with an incidence of 11.4 per cent in an equal number of patients with benign lesions of these organs.

The significance of these consistent differences in carbohydrate metabolism in cancer patients is not clear except to suggest the possible value of a more intensive study of the hormonal environment in which cancer develops.

It has been frequently stated that diabetes is more common in patients with carcinoma of the pancreas. In 609 necropsied cases of pancreatic carcinoma Bell (1957) found only a slightly but not significantly higher incidence of diabetes compared to 50,000 total necropsies studied in the same hospital. The incidence of cancer of the pancreas, however, in patients with existing diabetes was twice as frequent as in nondiabetic subjects. One out of every 7 carcinomas in diabetic patients was pan-

creatic in origin. Neither the location in the pancreas nor the extent of involvement of the tumor could be correlated with the presence or absence of diabetes.

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Chapter 48

HYPOGLYCEMOSIS

Robert H. Williams

GLUCOSE HOMEOSTASIS IN NORMALS

Normally, the concentration of glucose in the plasma is a balance between the amount contributed to it from the gastrointestinal tract and liver and that removed for storage or consumption by tissues. There are many factors that influence this balance and some of these are now considered individually under three major divisions: (1) effect on glycemia of amount, nature, and transformations of food, (2) effect of hormones on glycemia, and (3) direct effect of glycemia on hepatic glucogenesis.

Effect on Glycemia of the Amount, Nature, and Transformations of Food

ABSORPTION OF HEXOSES FROM THE GASTROINTESTINES. Cori demonstrated that there was a linear correlation of rat body weight with the amount of hexoses absorbed from rat gastrointestines (Fig. 48-1A), when the sugars were administered as 50 per cent solutions. The rate of absorption was independent of the total amount of hexose and its concentration. For example, even after absorption of 70 per cent of the hexose originally introduced, the rate of absorption was not diminished; 25, 50, and 80 per cent solutions were absorbed at the same rate. This

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The decreased liver glycogen probably was caused by increased glycogenolysis and decreased liver uptake of glucose during hypoglycemia. Although glucose is absorbed from the intestines twice as fast as fructose, similar amounts of liver glycogen were formed from equal doses; much less was formed from galactose. A maximal glycogen concentration in the liver was attained by 4 hours after glucose administration.

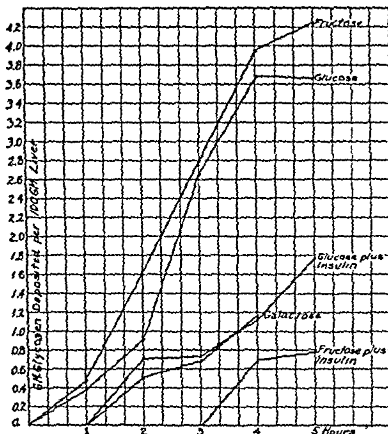


FIG. 48-1B. Rate of glycogen formation in the liver of normal and insulinized rats given hexoses by gavage. (After Cori, C. F.: *J. Biol. Chem.* 70 577, 1926.)

The amount of energy stored depends upon the extent of immediate need for it. With exercise, exposure to cold, and other situations associated with hypermetabolism, immediate oxidation of glucose will diminish its storage. These increased requirements also increase the rate at which fat, protein, and carbohydrate intermediates are converted to glucose. The extent to which fat can be converted to glucose has not been clearly established (Chap. 16), but apparently is slight. On the other hand, more than 90 per cent of ingested glucose that is stored may be stored as fat.

rate-limited absorption is apparently due to enzymatic activity proceeding at a maximal level. The order of the rate of absorption was galactose > glucose > fructose > mannose. Hypertonic sugar solution was diluted in the stomach with water osmosis.

HEXOSE UTILIZATION AND TRANSFORMATIONS. Studying rats, Cori reported that 90 per cent of glucose absorbed in 4 hours was oxidized or

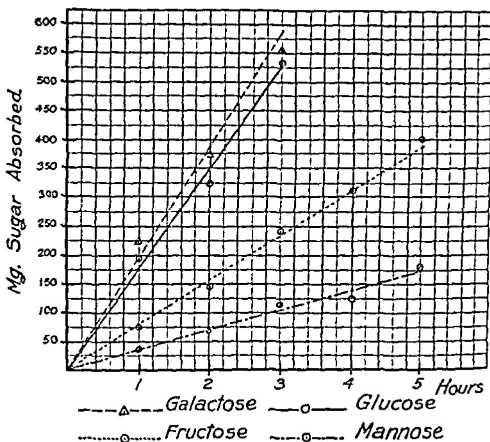


FIG. 48-1*A*. Linear rate of absorption from gastrointestines of hexoses per 100 gm. rat body weight per hour. (After Cori, C. F.: *J. Biol Chem.* 66:691, 1925.)

stored as glycogen, with the latter predominating unless insulin was injected. The tissues presumably handle glucose maximally with the ordinary glucose tolerance test because the rate at which glucose can be infused intravenously without causing glycosuria is not much greater than the rate at which glucose is absorbed from the intestine. Cori found that nonhepatic tissues stored more than twice as much glycogen as liver; with insulin the difference was much greater. Insulin decreased hepatic glycogen (Fig. 48-1*B*) and increased muscle glycogen.

The decreased liver glycogen probably was caused by increased glycogenolysis and decreased liver uptake of glucose during hypoglycemia. Although glucose is absorbed from the intestines twice as fast as fructose, similar amounts of liver glycogen were formed from equal doses; much less was formed from galactose. A maximal glycogen concentration in the liver was attained by 4 hours after glucose administration.

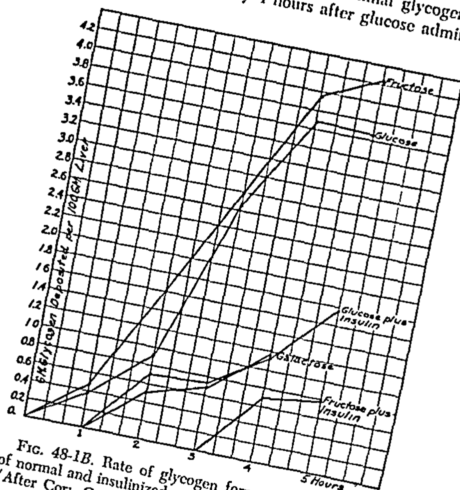


FIG. 48-1B. Rate of glycogen formation in the liver of normal and insulinized rats given hexoses by gavage. (After Cori, C. F.: *J. Biol. Chem.* 70:577, 1926)

The amount of energy stored depends upon the extent of immediate need for it. With exercise, exposure to cold, and other situations associated with hypermetabolism, immediate oxidation of glucose will diminish its storage. These increased requirements also increase the rate at which fat, protein, and carbohydrate intermediates are converted to glucose. The extent to which fat can be converted to glucose has not been clearly established (Chap. 16), but apparently is slight. On the other hand, more than 90 per cent of ingested glucose that is stored may be stored as fat.

Additional energy derived from glucose is stored also as protein. Energy may be derived from these foods as needed, but almost all gluconeogenesis is derived from protein and carbohydrates. Many tissues participate in the transformations of energy, but the liver plays the most

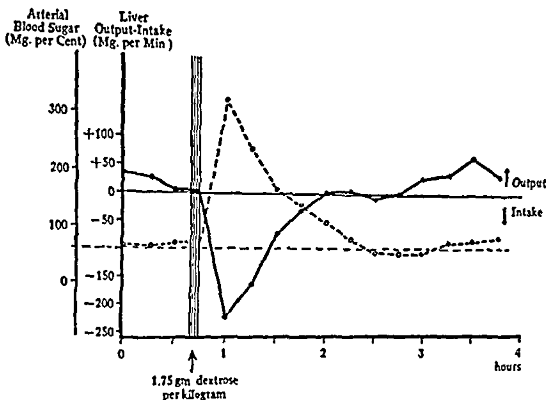


FIG. 48-2A. Effect of glucose administration upon the output and intake of sugar by the liver of an intact dog, calculated from blood sugar values and measurements of hepatic blood flow. The broken line represents arterial blood sugar values; the heavier, continuous line represents output or intake of sugar by the liver in milligrams per minute. There was a prompt cessation of sugar output when glucose was administered and a large intake of sugar followed Throughout the second hour the liver neither retained or excreted sugar. (After Soskin, S., and Levine, R.: *Carbohydrate Metabolism*, 2nd ed., Univ. of Chicago Press, 1952.)

important role. Since the activities of the liver, as well as those in extrahepatic tissue, are influenced markedly not only by the amount and nature of food available, but also by many different hormones, some of these individual influences are now considered.

AMOUNT AND NATURE OF FOOD. The type and amount of food ingested exerts many significant influences on the level of blood glucose and on chemical transformations; the mechanism in some instances is unknown.

Kuzuya has shown in dogs that the administration of glucose or equicaloric amino acids or fat causes a severalfold increase in plasma insulin activity. Indeed, he also showed that the continuous injection of a small amount of glucose into the femoral vein increased plasma insulin activity. On the other hand, glucose injected into the pancreaticoduodenal artery in very small quantity, yet sufficient to cause more

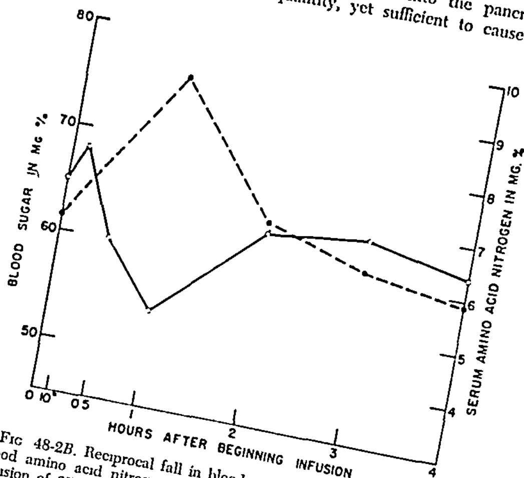


FIG 48-2B. Reciprocal fall in blood sugar (solid line) with rise in blood amino acid nitrogen (broken line) following the intravenous infusion of 250 ml. of 10 per cent amino acids into subjects without disease involving glucose metabolism. (After Mellinkoff *et al.*, *Stanford M. Bull.* 13:117, 1955.)

hyperglycemia in the pancreas than in the aforementioned experiment dealing with intrafemoral injection, did not cause an increase in plasma insulin activity. Moreover, stimulation of the splanchnic nerve caused hyperglycemia in the pancreas but no increase in plasma insulin activity. No increase in plasma insulin activity followed the administration of glucose, amino acids, or somatotropin in animals with section of the right vagus nerve; section of the left vagus was immaterial.

Kuzuya concluded that the food, working on the central nervous system, increased insulin secretion via right vagal stimulation of the β-cells. His conclusions seem to have good basis, but observations by some investigators have suggested that under certain conditions, hyperglycemia can directly stimulate the pancreas to secrete insulin.

Glucose administration has been reported by Soskin to decrease hepatic glucogenesis (Fig. 48-2A). It has not been determined how much of the change is due to a direct effect on the liver, as discussed later, and how much is related to various indirect changes induced by hormones or other influences.

Ribose causes hypoglycemia in man, but the mechanism has not been elucidated. Protein administration exerts variable influences. Casein hydrolysate has been shown by Mellinkoff to produce hypoglycemia (Fig. 48-2B), probably by increasing insulin secretion. Greenstein, *et al.*, reported that individual amino acids exert different effects; certain ones, for example, leucine, isoleucine, and tryptophane in large doses in rats produce death from hypoglycemia. Whereas arginine also produces hypoglycemia, though less severe, it offers some protection from the hypoglycemic action of leucine or tryptophane. Tryptophane inhibits significantly the degradation of insulin by liver "insulinase" but leucine does not. L-Amino acids are more hypoglycemic than the D- form.

Amino acids are glycogenic to varying degrees. Many of the transformations of food are related not only to the amount of available carbohydrate but also to its utilizability. Several hormones play a major role in these transformations.

Effect of Hormones on Glycemia

CORTICOSTEROIDS. Certain adrenal steroids, especially hydrocortisone, help to prevent hypoglycemia, particularly during fasting. Hydrocortisone stimulates new glucose formation, mainly from protein in the liver. It increases the storage of glycogen in the liver and also increases glucose-6-phosphatase activity, permitting faster release of glucose. It, in conjunction with somatotropin, antagonizes the action of insulin, thereby *decreasing utilization of glucose in many tissues.*

SOMATOTROPIN. Somatotropin influences the blood glucose concentration in several ways (Chap. 17): It affects the amount of insulin secreted, its fate, and its action. It has been reported to: (1) increase the secretion of insulin (with prolonged administration of large doses it presumably causes a decrease, owing to β-cell exhaustion), (2) increase glucagon secretion (not definitely established), (3) competitively inhibit insulin and glucagon degradation by liver enzyme preparation in vitro, (4) cause adipolysis and proteogenesis, (5) inhibit the

hexose-monophosphate shunt, (6) increase liver glycogen and responsiveness to hypoglycemia and (7) antagonize insulin action. As discussed in Chapter 31, somatotropin in conjunction with hydrocortisone is apparently responsible for the development of insulin antagonistic factors in serum, found in the lipoprotein fraction and albumin fraction. Glucagon, unlike epinephrine, does not produce glycogenolysis in muscle and apparently does not directly affect glucose utilization by tissues; there are some reports with claims that glucagon increases glucose uptake.

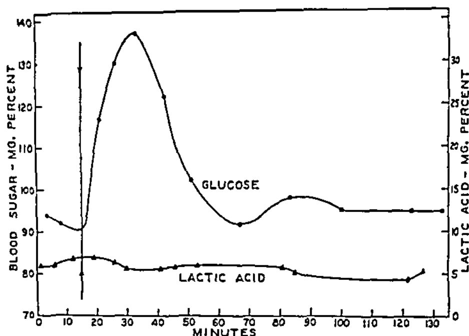


FIG. 48-3A. Blood sugar and lactic acid levels following the intravenous injection of glucagon into a rabbit. (After Sutherland, E. W.: *Recent Progress in Hormone Research* 5:441, 1950, New York, Academic Press.)

GLUCAGON. The major action of glucagon (hyperglycemic factor—HGF), presumably is to promote hepatic glucogenesis by increasing glycogenolysis (Fig. 48-3A). This it effects by increasing phosphorylase activity. Preliminary studies have shown that glucagon inhibits lipogenesis and proteogenesis.

EPINEPHRINE. Epinephrine promotes glycogenolysis not only in the liver but also in the muscles (Fig. 48-3B) (Chap. 15). The extent to which epinephrine leads to hepatic glucogenesis depends upon the availability of glycogen. With disease of the liver the response is diminished (Fig. 48-3C). Epinephrine increases gluconeogenesis by increas-

ing adrenocorticotropin secretion and adrenal corticoids; glucagon increases gluconeogenesis in the absence of the adrenals. Epinephrine inhibits glucose utilization (Fig. 48-3*D*), presumably by producing an increase in glucose-6-phosphate and inhibition of glucokinase. Ordinarily the higher the arterial blood sugar, the greater is the rate of glucose utilization. When, however, the hyperglycemia is attained via

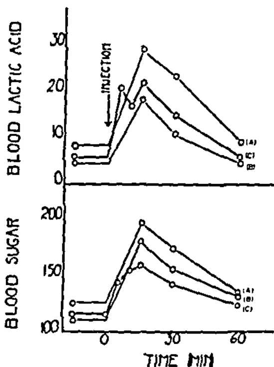


FIG. 48-3*B*. Increase of blood sugar and lactic acid of normal rabbits injected suddenly intravenously with 0.03 mg. of epinephrine per kilogram. (After Cori, C. F., *et al.: Am. J. Physiol.* 93:273, 1930.)

epinephrine the amount of glucose utilization may be markedly subnormal.

It is difficult to state the extent to which glucose utilization by the brain is influenced by epinephrine and norepinephrine. There are observations reported indicating that under certain conditions they decrease utilization and under others increase it.

INSULIN. Insulin exerts powerful lipogenetic and proteogenetic actions. It also increases glucose uptake and utilization by many nonhepatic tissues. Under certain circumstances it may increase or de-

crease the glucose uptake and utilization by liver. For example, in untreated diabetics it increases glucose uptake and utilization and in normals given a large dose the reverse effect may be obtained. It decreases glucose-6-phosphatase activity which, of course, tends to decrease hepatic glucogenesis, particularly in diabetics. In normals, certain doses

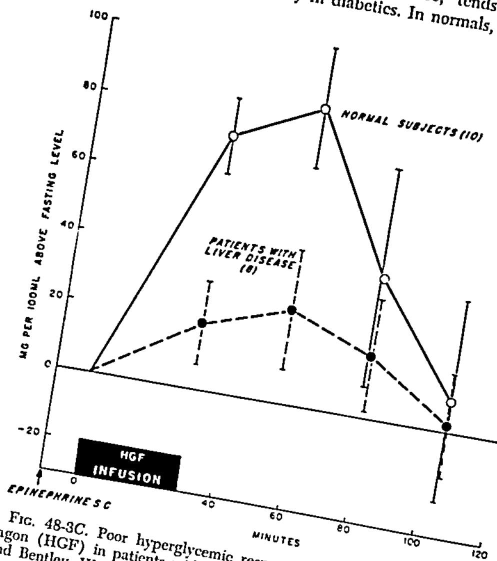


FIG. 48-3C. Poor hyperglycemic response to epinephrine plus glucagon (HGF) in patients with liver disease. (After Van Itallie, T. B., and Bentley, W. B. A., *J. Clin. Invest.* 34:1730, 1955.)

of insulin have been found not to affect hepatic glucogenesis. The extent to which insulin lowers the blood glucose concentration depends upon many factors, particularly hormones and antagonistic substances in the serum. With the blood glucose approaching 50 mg. per 100 ml. a series of defensive reactions are initiated, supposedly consisting of (1) direct stimulation of liver glucogenesis by hypoglycemia (discussed

later), (2) increased sympathicotonia, (3) hyperepinephrinemia, (4) hyperglucagonemia, (5) hypercorticoidism, and (6) hypersomatotropinism. The evidence for some of these changes is meager.

Cannon demonstrated many years ago that hypoglycemia increased epinephrine secretion. Hypoglycemia stimulates various areas of the

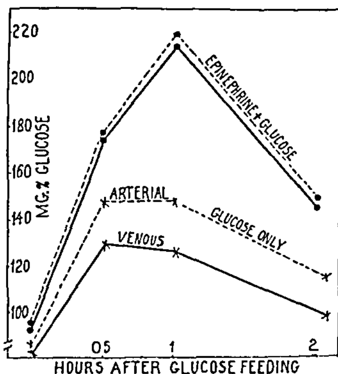


Fig. 48-3*D*. Composite curves showing the arterial (capillary) and venous blood sugar curves after the ingestion of 50 gm of glucose with and without the simultaneous injection subcutaneously of 0.2 mg of epinephrine. The markedly decreased A-V difference is caused by epinephrine, even at a much greater sugar concentration. (After Somogyi, M.: *J Biol Chem.* *186*:513, 1950.)

brain, including the hypothalamus, pons, and medulla. Impulses are sent through the sympathetic nervous system to the adrenal medullae, increasing the secretion of epinephrine and norepinephrine. Section of the splanchnic nerves prevents the discharge of epinephrine. One hour after injection in fasting subjects of sufficient insulin to produce hypoglycemia, there is a depletion of adrenal ascorbic acid and cholesterol,

together with lymphopenia. Although epinephrine can increase corticotropin secretion and thereby increase corticoid production, it has been shown that some increase in corticoid secretion occurs even without epinephrine stimulation, because the lymphopenia occurs with hypoglycemia in adrenal demedullated animals. Moreover, Goldfien (Fig. 48-4) found an increase in plasma 17-hydroxycorticosteroids before

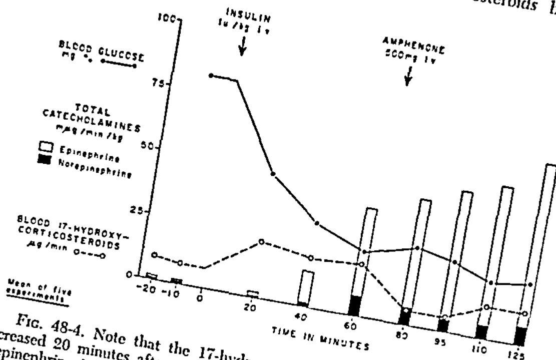


FIG. 48-4. Note that the 17-hydroxycorticosteroid plasma level had increased 20 minutes after insulin administration. The epinephrine and norepinephrine levels did not increase until later. Amphenone administration decreased the 17-hydroxycorticosteroids but not the epinephrine nor norepinephrine. (After Goldfien, A., *et al.*: *Endocrinology 62*:749, 1958.)

there was an increase in epinephrine. The evidence for hypoglycemia-induced increased somatotropin and glucagon is not direct but suggestive. There is clearly an increased sympathicotonia and at least part of this occurs before there is demonstrable hyperepinephrinemia. When 6 units of insulin are injected intravenously into a hyperglycemic subject, or in adrenalectomized or hypophysectomized subjects, a progressive decline in blood sugar may occur for several hours (Fig. 48-5A) because crystalline insulin exerts an action for at least 6 hours. However, when the same amount of insulin is injected into fasting normals, a nadir is reached in approximately 30 minutes, and normoglycemia is attained by 2 hours. This is because of the mobilization of many forces

antagonistic to insulin. The marked influence of these forces is also demonstrated by Somogyi's studies, shown in Figure 48-5*B*. It is clearly evident that the same individual reacts quite differently to glucose administration after a hypoglycemic reaction has mobilized many homeostatic mechanisms. When insulin is given simultaneously with the glu-

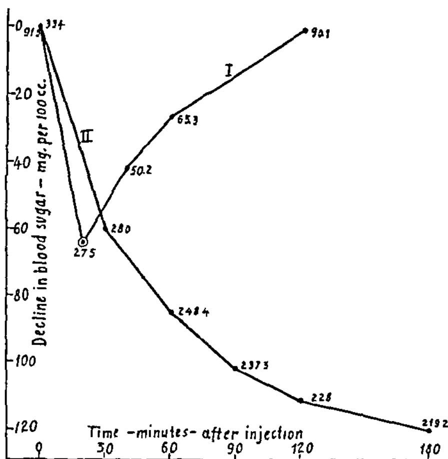


FIG. 48-5*A*. A diabetic subject injected intravenously with 6 units of insulin showed a progressive decline in blood sugar (Curve II), but 3 healthy subjects with a normal fasting blood sugar showed a sharp rise soon after a decline of 60 mg. per 100 ml. (Curve I). The figures along the two curves are the actual blood sugar values The figures on the ordinate show the decline from the fasting level. (After Somogyi, M.: *J. Biol. Chem.* *179*.217, 1949.)

cose there is an increased A-V glucose difference but when the insulin is given 40 to 50 minutes previously the A-V difference is markedly decreased; indeed, little glucose is used; marked hyperglycemia, glycosuria, and ketonuria may develop. In general, the higher the blood sugar level, the greater is the A-V glucose difference and the more

glucose utilized, but when insulin antagonistic forces have been mobilized there may be relatively little glucose utilization even at high blood sugar levels. As shown in Figure 48-5C, 6 units of insulin may actually cause less glucose utilization than 3 units. In general, the greater the

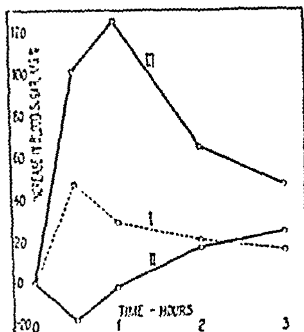


Fig. 48-5B. Glucose tolerance curves obtained after 100 gm. of glucose was administered orally to a healthy man on three different occasions. Curve I reveals the changes in blood glucose after administration of glucose alone. Curve II was obtained when 5 units of insulin were injected intravenously simultaneously with glucose, and Curve III was obtained when the same dose of insulin was injected 40 minutes before glucose. The enormous decrease in glucose tolerance resulting from insulinogenic hypoglycemia is impressive. (After Somogyi, M.: J. Biol. Chem. 193:859, 1951.)

hypoglycemia the less glucose utilized. This decreased peripheral glucose utilization and accompanying increased hepatic glucogenesis help restore normoglycemia.

These observations help explain the perplexing problem of brittle diabetes and the marked variations in the results of glucose and insulin tolerance tests conducted in patients with insulinomas. It is clearly evi-

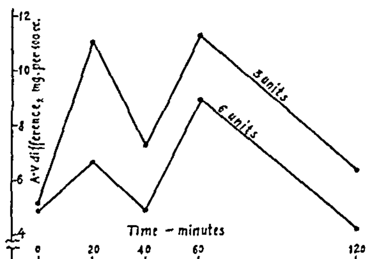


FIG. 48-5C. Shows that in a healthy man 6 units of insulin may promote less glucose utilization than 3 units. (After Somogyi, M.: *J. Biol. Chem.* *193*:859, 1951.)

dent that the curves will vary tremendously with the periodic secretion of insulin into the blood stream.

Direct Effect of Glycemia on Hepatic Glucogenesis

Lazarus and Volk have collected evidence suggesting that the mechanism for responsiveness to hypoglycemia is a direct response of the liver, via glycogenolysis, and that the endocrine factors make their major contribution by (1) helping to supply liver glycogen and (2) by determining the blood sugar level at which responsiveness to hypoglycemia will occur. As partially shown in Figure 48-6, responsiveness to hypoglycemia was observed in animals in all the following conditions: (1) somatotropin-pretreated, adrenalectomized; (2) ACTH-treated, hypophysectomized; (3) adrenodemedullated; (4) pancreatectomized; (5) sympathectomized; or (6) treated with various autonomic blocking agents. Soskin and Levine also stated that the fundamental regulation of the blood sugar depended on the autoregulation induced by the blood sugar level itself. As the blood sugar concentration increases there is decreased hepatic glucogenesis and increased insulin secretion, leading to increased removal of glucose from the blood; with decrease in the blood sugar (within certain limits) there is an increase in hepatic glucogenesis and a decrease in insulin secretion. Thus hypoglycemia can be visualized as triggering hepatic glucogenesis in a liver prepared for this with the assistance of hormones and numerous other factors.

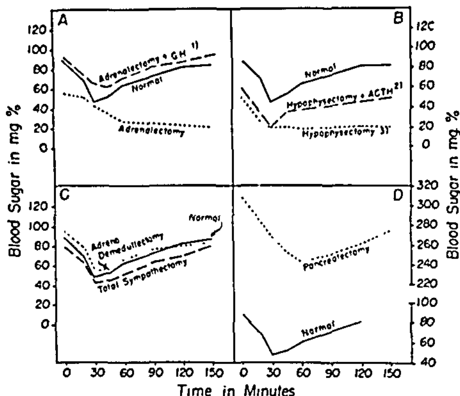


Fig. 48-6. The results of four sets of experiments are presented in panels A, B, C, and D. The mean blood sugar values after the intravenous administration of 0.1 units of insulin per kilogram of dog, show that the pituitary, the adrenal cortex or medulla, the pancreas, and the sympathetic nervous system are not indispensable for hypoglycemia responsiveness. (1) Pretreated with 1 mg. somatotropin per kilogram. (2) Pretreated with 50 mg. of ACTH daily for 3 weeks after hypophysectomy. (3) Same as (2), 3 weeks later. (After Lazarus, S. S., and Volk, B. W.: *Metabolism* 2.500, 1953.)

Summary

The quantity and type of food ingested significantly influence blood glucose concentrations. In some instances the action is direct and in others via hormones. As shown in Table 48-1, many hormones influence liver glucogenesis, glucose utilization, insulin secretion, and glucosteroid secretion. The main duty of insulin is to promote transfer of glucose through the cell wall of many tissues. However, there are many different agents that can antagonize this action (Fig. 48-7). Enzyme systems that inactivate insulin exist in various tissues, particularly the liver. Hydrocortisone promotes hepatic glucogenesis and, working in conjunction wtih somatotropin, it antagonizes insulin action by leading to an accumulation of (1) a lipoprotein factor that binds insulin, (2)

TABLE 48-1. EFFECTS OF HORMONES ON GLUCOSE *PRODUCTION AND UTILIZATION*

	Liver glucogenesis	*Glucose utilization*	*Insulin secretion*	*Glucosteroid secretion*
Growth hormone	+*	−†	+	+
Glucosteroids	+	−	+	−
Epinephrine	+	−	+	+
Glucagon	+	±	+	+
Insulin	−	+	−	+
Thyroxin	+	+	+	+

* + = increase.
† − = decrease.
Note: A few of the changes are observed only under certain conditions (see text).

a factor in albumin that antagonizes the action of insulin. Under certain conditions, particularly ketoacidosis, a factor accumulates in the α-globulin fraction that blocks insulin action. With repeated administra-

FIG 48-7. See text. The activities apply chiefly to extrahepatic cells, except for the glucagon, epinephrine, and hydrocortisone glucogenic actions.

tion of insulin, antibodies to it accumulate which, complexed to insulin, migrate upon electrophoresis to the β-γ-globulin interzone. Periodically, insulin is released from the protein complex, and occasionally produces hypoglycemia. Glucagon and epinephrine each promotes hepatic glucogenesis. Epinephrine and ketone bodies hinder glucose utilization.

Within a few minutes after the development of hypoglycemia the following changes probably are initiated:

1. Decrease in glucose utilization resulting from:
   a. Decrease in mass action of glucose on its own transfer into cells.
   b. Increased inhibition of insulin action by glucosteroids. (This action is augmented by growth hormone, but it seems to require several hours to increase this activity of growth hormone.)
   c. Inhibition by epinephrine of glucose utilization.
   d. Decreased insulin secretion.
2. Increase in hepatic glucogenesis resulting from:
   a. Direct effect of hypoglycemia on the liver.
   b. Increased secretion of glucagon.
   c. Increased sympathicotonia.
   d. Increased secretion of epinephrine.

Since no significant increase in liver glycogen occurs in rats in less than 2 hours following the intravenous administration of a large quantity of hydrocortisone or the subcutaneous administration of alanine, it is probable that not much increase in gluconeogenesis occurs during the responsive period to hypoglycemia. However, the amount of gluconeogenesis that has occurred immediately preceding the test and persists during the test is very important, since it largely determines the amount of the liver glycogen stored in the subject fasted overnight.

When 100 gm. of glucose is administered orally, as in a glucose tolerance test, a peak in blood sugar is observed about 30 minutes later. In normals the level then rapidly falls, but in diabetics the fall is much more gradual. In normals the hyperglycemia stimulates insulin secretion and inhibits hepatic glucogenesis, but in diabetics both of these changes are less marked.

PHYSIOLOGIC, PATHOLOGIC, AND CLINICAL HYPOGLYCEMIC CHANGES

Symptoms and Signs of Hypoglycemia and Mechanisms in Their Development

Most of the clinical manifestations of hypoglycemia emanate from the nervous system. Since the brain derives almost all its nutrition from

carbohydrate, since it stores very little glucose or glycogen, and since it has a very high energy requirement, significant decreases in its utilization of glucose may cause many symptoms, essentially those of anoxia. The brain has a high oxygen consumption: about 30 times greater than equivalent muscle mass; its respiratory quotient is approximately 1. The organs other than the brain use fat as well as carbohydrate for energy. Although with hypoglycemia there ordinarily is a normal oxygen saturation of the blood and a satisfactory blood flow, the results are essentially the same as though there were a low oxygen availability, because deficiency of glucose decreases oxygen utilization by the brain. Indeed, it must be emphasized that glucose *utilization* is the important factor and not blood glucose concentration per se. A number of biochemical agents or morphologic alterations may interfere with glucose utilization. Using a compound that blocks glucose utilization, deoxyglucose, it has been shown that clinical reactions characterizing hypoglycemia may occur with marked hyperglycemia. Moreover, as shown in Figure 48-3D, glucose utilization may be markedly impaired with hyperepinephrinemia, in spite of an associated pronounced hyperglycemia. Previous damage to the brain accentuates the hypoglycemic symptomatology by interfering with cerebral circulation. For example, previous experimental ligation of the middle cerebral artery in animals contributes to the development of hemiparesis or hemiplegia when hypoglycemia is produced. The same phenomenon is observed in patients with cerebral thrombosis, with alcoholic brain disease, and with many other disorders. Indeed, anything that produces anoxia or in any way interferes with the oxidation of glucose contributes to clinical hypoglycemic reactions. Moreover, the higher the glucose and oxygen requirements, the more likely is a given lowering of the blood sugar to produce symptoms; thus an adult has more symptoms than an infant since the latter has a lower requirement for cerebral glucose oxidation.

Although the true blood glucose concentration is usually found to be below 50 mg. per cent when there are clinical manifestations of hypoglycemia, there are exceptions to this. Moreover, there often is not a good correlation of the level of the blood sugar with the intensity of the clinical manifestations. This is because the blood sugar value is not a direct indicator of the rate of glucose utilization; cerebral A-V glucose differences and oxygen differences are much better indicators. These values may approach zero with severe hypoglycemic reactions. Moreover, following a severe hypoglycemic reaction the blood sugar level has been found to be hypernormal when the cerebral oxygen consumption was less than 25 per cent normal. Under certain conditions the administration of pure oxygen has been found to decrease the clinical

hypoglycemic manifestations, whereas factors that induce anoxia intensify them.

Most of the early symptoms are caused by mild cerebral anoxia and are associated with varying degrees of hyperepinephrinemic manifestations. Anoxia stimulates the sympathetic nervous system but depresses the somatic nervous system. The faster the blood sugar falls, the more pronounced are the hyperepinephrinemic features. They consist of

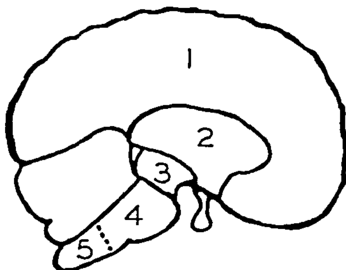


Fig. 48-8. The order in which different areas of the brain are affected by hypoglycemia is proportional to the needs for glucose and inversely correlated with the phylogenesis. (After Himwich, H. E.: *Brain Metabolism and Cerebral Disorders*. Baltimore, The Waverly Press, 1951.)

weakness, sweating, tachycardia, anxiety, nervousness, tremor, and hunger. To these may be added certain cerebral manifestations that predominate when the hypoglycemia develops gradually. They consist of headache, visual disturbance, mental confusion, and other reactions as now described.

Sequential hypoglycemic symptoms and signs were carefully studied by Frostig, Himwich, and others in schizophrenic patients subjected to insulin shock therapy. The changes were described as progressively involving 5 different areas of the brain (Fig. 48-8) in reverse order of their phylogenesis and their oxygen requirement. The cerebral hemi-

spheres and parts of the cerebellum are involved first. This phase continues until the blood sugar reaches its nadir. The second phase is associated with the release of activities in the subcortico-diencephalic area, which consists of 3 kinds of centers: subcortical motor nuclei, sensory thalamic nuclei, and autonomic or visceral nuclei. The third phase results from liberation of the midbrain, and the fourth phase frees the functions regulated by the upper part of the medulla oblongata. The fifth phase is due to the release of the lower part of the medulla oblongata and its vital centers, respiratory and cardiovascular.

The major manifestations of hypoglycemia are listed in Table 48-2.

TABLE 48-2. SYMPTOMS AND SIGNS OF DIFFERENT PHASES OF HYPOGLYCEMIA*

Phases†	*A-V O_2 diff.*	*Symptoms and signs*
Cortical	6.8	Somnolence, perspiration, hypotonia, tremor.
Subcortico-diencephalic		Loss of consciousness; primitive movements (sucking, grasping, grimacing), twitches, restlessness, clonic spasms, hyperresponsiveness to pain, sympathicotonia (tachycardia, erythema, perspiration, mydriasis).
Mesencephalic	2.6	Tonic spasms, inconjugate ocular deviation, Babinski sign
Premyencephalic		Extensor spasms. Rotation of head causes extensor spasm of extremities on the side toward which the chin points and flexor spasm on the opposite side.
Myencephalic	1.8	Deep coma, shallow respiration, bradycardia, miosis, no pupillary reaction to light, hypothermia, atonia, hyporeflexia, absent corneal reflex.

* After H. E. Himwich, *Brain Metabolism and Cerebral Disorders*. Baltimore, Waverly Press, Inc., 1951.

† With insulin shock therapy and in some patients with insulinoma all 5 phases are induced in sequence, in those with "functional" hypoglycemia no more than the first 2 phases tend to develop.

They all are reversible if the low level of glucose oxidation does not persist too long. If glucose is administered before the fifth phase has lasted for 15 minutes, all the disturbances disappear, in reverse order of their appearance. The vagotonic features disappear immediately. The extensor spasms of the fourth phase last for a few moments, and then are replaced by the tonic spasms of the third phase. The restlessness of the second phase follows directly and consciousness is gradually regained. If administration of carbohydrate is delayed, hours or days may be required for recovery, or permanent damage to the nervous system may follow. Coma has been found in dogs to persist for 24 hours

after only 4 minutes of arrest of brain circulation. Following coma there is a transition period lasting several days, characterized by gradually improving function of the cerebral cortex and of the postural righting mechanisms, as well as by severe ataxia of the cerebellar type. A brain that is completely anoxic for a few minutes tends to be irreversibly damaged. Persistence of hypoglycemia for a few minutes after disappearance of the corneal reflex may be associated with permanent brain damage. In cats, Tyler found that brain damage did not occur unless the medullary phase was maintained for more than 100 minutes; the lower was the body temperature, the longer it took to produce brain damage. With insulin shock therapy, coma is usually stopped in 90 minutes; full consciousness is attained 30 minutes later.

Many changes in the electroencephalogram are observed with hypoglycemia, but the electrical changes correlate much better with cerebral oxygen utilization than with the blood sugar. With intensification of the reaction the α-waves disappear and are replaced by δ-waves. By the time unconsciousness develops all α-waves have disappeared; they reappear with regaining consciousness.

Permanent Sequelae from Hypoglycemia

A large variety of permanent neurologic disorders and patterns of psychobiologic disintegration follow severe hypoglycemic reactions. Among the group are: mental deterioration (see case report later), paraergasia (schizophrenia), thymergasia (affective disorders), hemiparesis or hemiplegia (often transient), aphasia, choreiform movements, Parkinsonism, epilepsy, and narcolepsy. Death may occur within minutes, days, or months.

Although hypoglycemia causes most of its damage in the brain, it produces a significantly increased incidence of angina and myocardial infarction, particularly in older diabetics.

Pathologic Brain Changes

Baker, Courville, and others have reported many changes in the nervous system following severe hypoglycemia. They are the same ones that result from anoxia, induced in a variety of ways. Anoxia, in addition to damaging the nerve cells in general, causes vasospasm with resulting spotty ischemic necrosis. The vasomotor center especially is involved. The most marked changes tend to be found in the cerebral cortex, the basal ganglia (particularly the corpus striatum), and the hippocampus. Especially in the acute stage there are scattered petechiae, congestion, and swelling of the nerve cells. These undergo a variety of degenerative changes and many of them disappear, particularly in th

laminar zone. Numerous areas of glial reaction are observed. There is *demyelination, encephalomalacia* and, *sometimes, cyst formation.* Peripheral nerve degeneration may also be observed.

Detailed considerations involved in the clinical diagnosis of the hypoglycemoses are presented later.

ETIOLOGIES OF HYPOGLYCEMIAS

Hypoglycemia may be caused by many different factors, as indicated in Table 48-3. There is disagreement among authors as to the maximal

TABLE 48-3. ETIOLOGIES OF HYPOGLYCEMIA

I. Deficient food supply
  a. Starvation
  b. Excess elimination from body (milk, urine, stools)
  c. Hyperutilization (exercise, fever, thyrotoxicosis, neoplasms)
  d. Abnormal types of metabolism (galactosemia, familial aminogenic hypogly- [illegible]
II. [illegible]
  [illegible]
  b. [illegible]
    4. Hypoglucagonemia (?)
    [illegible]
III. [illegible]
  [illegible]
  c. Posthyperglycemia neurogenic (right vagus)
  d. Tachyalimentation (posthyperhyperglycemia)
IV. [illegible]
V. Pharmacogenic or toxigenic compounds
  a. Excess insulin
  b. Other agents—e.g., biguanides, guanidines, sulfonylureas, hypoglycin, amino acids, somatotropin (early), prolactin, alloxan (early), hydrazine, Antistine, Antergan, Neoantergan, Pyribenzamine.
VI. Idiopathic hypoglycemia
  a. Infantile (McQuarrie's syndrome)
  b. Early diabetes

blood sugar level permissible for the diagnosis of hypoglycemia. A level below 40 mg. per 100 ml., using a true blood sugar method, or below 55 mg. with a method that includes reducing nonsaccharoids, seems to be satisfactory. However, it must be emphasized, as discussed pre-

viously, that a hypoglycemic reaction may occur at higher levels or may not occur at levels much lower than these.

DEFICIENT FOOD SUPPLY

STARVATION. A deficiency in the supply of food can be produced in many different ways (Table 48-3). In all, there is a decrease in the total amount of food stored in the body. Therefore, fasting tends to produce hypoglycemia in each type listed. The normal adult has approximately 300 gm. of glycogen in muscle, 150 gm. of glycogen in the liver, and less than 20 gm. of glucose in the extracellular fluid. All the carbohydrate would supply energy for less than 2 or 3 days. However, a normal subject usually can survive total starvation for weeks, because of the nutrition derived from fat and protein. Hypoglycemia may develop in starvation states, such as anorexia nervosa; indeed, in such situations the blood sugar has been found below 30 mg. per 100 ml. Mild hypoglycemic manifestations appear in some subjects with a 1-day or 2-day fast. The glucose tolerance is variable.

EXCESS ELIMINATION. Hypoglycemia has been observed in a number of conditions associated with marked loss of food supply from the body, as for example with lactation, gastrointestinal malabsorption (diarrhea, steatorrhea, gastrointestinal resection), food wastage in urine (glycosuria, hyperaminoaciduria, and proteinuria).

HYPERUTILIZATION. Hypoglycemia is also observed with conditions associated with hypermetabolism, for example, severe exercise, fever, exposure to cold, and thyrotoxicosis. In this category falls the hypoglycemia observed with neoplasms, particularly large fibrosarcomas (Scholz, *et al.*). These tumors may consume an enormous amount of glucose. Whereas extracts of some such tumors have contained hypoglycemic material, most of them have not.

ABNORMAL METABOLISM. Derangements in metabolism that produce hypoglycemia are being recognized with increasing frequency. One of these is galactosemia, which is associated with hepatosplenomegaly, jaundice, cataracts, mental deficiency, and galactosuria. The manifestations are intensified by milk ingestion and ameliorated by its avoidance. With this disorder there is a congenital deficiency in P-gal (uridyl) transferase, leading to a failure to form glucose-1-P from galactose-1-P (Kalckar, *et al.*). Since there seems to be no problem in converting glucose to galactose, some of the food energy is sidetracked and much of it is wasted in the urine as galactose. Another hypoglycemic entity associated with a congenital metabolic defect has been found by Cochrane, *et al.*, to be familial and precipitated by the administration of protein, leucine, or isovaleric acid. There is a significant

increase in blood nonsugar reducing substances, supposedly mainly glutathione and ergothioneine. Oral doses of leucine and isovaleric acid that cause hypoglycemia with this familial disorder were found to have no effect on the blood sugar of normals. However, leucine in much smaller dosage, when injected subcutaneously, has been reported to produce significant hypoglycemia in normals; this is difficult to match with the previously discussed observations of Cochrane, *et al.* Moreover, as discussed earlier, many amino acids have been found to produce hypoglycemia. The mechanism may involve increased secretion or decreased degradation of insulin; another possible mechanism is decreased hepatic glucogenesis. Since there has been demonstrated a considerable variation in the blood sugar response (hypoglycemia or hyperglycemia) to different amino acids, variation in response in different subjects and variation in response under different conditions in the same subject, careful observations of the effect of protein administration on the blood sugar must be made in each patient with hypoglycemosis. Some hypoglycemoses benefit from a high protein diet whereas others, such as the above described familial type, may be intensified by a high protein intake, especially one high in leucine and its degradation product, isovaleric acid.

Any one of a large number of factors that produce a state of anaerobiosis increases the uptake of glucose by tissue and hypoglycemia may result.

HEPATOGENIC DISORDERS

Primary Hepatogenic Disorders

Many different types of disorders can lead to decreased hepatic glucogenesis, causing hypoglycemia (Table 48-3). In each instance there is too little formation of glucose precursors or there is hypoglycogenolysis. In some there is a decreased uptake by the liver of glucose and other food constituents, whereas in others the uptake may be increased. There tends to be a decreased hyperglycemic response to epinephrine or glucagon (Chap. 30). Frequent feedings of a high protein and high carbohydrate diet tend to be beneficial and corticosteroid therapy aids many patients. Correction of the primary defect should be carried out when possible.

GENERAL HYPOFUNCTION. Removal of the liver rapidly leads to death from hypoglycemia unless glucose is supplied almost continuously. Extensive, severe liver disease also is known to produce hypoglycemia, but not very commonly. For example, [illegible] *al.*, in studying 269 patients with [illegible] 'vement [illegible] [illegible]osis, carcinoma, or

liver and kidney, which is responsible for the dephosphorylation o! cose, permitting its liberation into the blood. As a consequence, cogen accumulates excessively in the liver and kidney; it may als crease in the blood. There may be impaired growth, hyperlip(acetonuria, and decreased serum phosphorus.

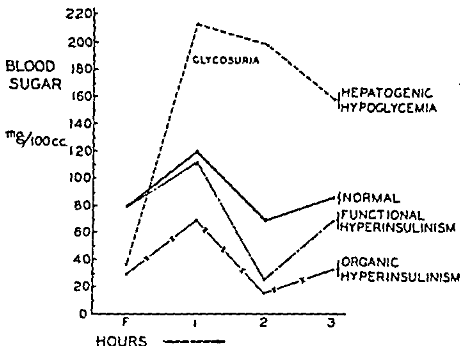


FIG 48-9. Glucose tolerance curves in different types of hypoglycemoses. Note that the fasting level is normal with "functional hyperinsulinism" (this is the same that I designate as "posthyperglycemia neurogenic hypoglycemia"), but is subnormal with the hepatogenic and organic hyperinsulinism. There is persistent hypoglycemia with organic hyperinsulinism and postprandial hypoglycemia with "functional hypoglycemia." (After Conn, J. W., and Seltzer, H S · *Am. J. Med.* 19:460, 1955.)

2. *Glycogen Storage Disease of the Heart.* With this type, the heart contains enormous quantities of glycogen. The heart is greatly enlarged and there often is congestive heart failure, with death in the first few months of life. Many striated muscles also contain large glycogen stores. Weakness and listlessness are common. The ... markedly enlarged. Excess glycogen deposits ... d reticuloendothelial system, kidney, li ... , t and bone marrow It is also found ... intestinal tract, acini and islets of p ... and in the nervous system. The c...

emonstrated, but it is not due to a deficiency of glucose-6-phosphatase.

3. *Diffuse Glycogenesis with Hepatic Cirrhosis.* This disorder presumably results from a deficiency in an enzyme that debranches glycogen. Consequently, glycogen of an unusual type (fewer branches than normal and longer chains) accumulates in the liver, spleen, lymph nodes, nd scattered lymph follicles. This compound seems to be irritating to issues; surrounding the glycogen deposits are accumulations of fibrous issue. Indeed, in the liver there is frank cirrhosis, with hepatosplenomegaly, ascites, jaundice, and anemia. Death usually occurs in he first few years of life.

4. *Glycogen Storage of Liver and Muscle.* This very rare type also eems to be associated with subnormal function in glycogen debranching activity, leading to the accumulation, particularly in liver and muscle, of glycogen with an excessive number of branches and very short outer chains. Apparently, the outer branches are readily released and rebuilt, but the inner core is unavailable as a source of glucose.

The second group has a normal hyperglycemic response to epinephrine, but the other 3 groups tend to show subnormal responses. Hypoglycemia, ketosis, and hyperlipemia occur with the first and fourth types. The glycogen structure is normal in the first 2 types, but abnormal in the others. Treatment is not very satisfactory. Frequent feedings with a high protein diet are beneficial; in addition, corticosteroid therapy has been helpful in some cases. Death occurs early, except in children with the hepatic form. They may live a long time but there is retardation in somatic growth and sexual development. There is increased susceptibility to infection because of impaired leucocytic activity.

Secondary Hepatogenic Disorders

There are a number of disorders (Table 48-3), not primarily involving the liver, which produce hypoglycemia as a result of deficient stimulation of hepatic glucogenesis.

HYPOADRENOCORTICISM (ADDISON'S DISEASE). With deficiency in adrenal corticosteroids there is decreased gluconeogenesis. Especially during fasting this may lead to insufficient liver glycogen and decreased release of glucose from the liver, thereby permitting hypoglycemia to develop. These subjects have an increased sensitivity to insulin (Chap. 30), and decreased hypoglycemic responsiveness. The glucose tolerance test is characterized by a low fasting level and a hypoglycemic response in 2 or 3 hours. Therapy consists of frequent feedings of a high protein diet and corticosteroid therapy.

A few instances of hypoglycemia have been observed in congenital adrenal hyperplasia, associated with insufficient hydrocortisone syn-

thesis. Treatment consists of corticosteroid therapy and frequent feedings.

PANHYPOPITUITARISM (SIMMONDS' DISEASE). The pattern in this type and the therapy are similar to those of hypoadrenocorticism; in each there is deficiency of adrenocorticosteroids. In Simmonds' disease there *is also a deficiency of somatotropin, which also is important in hepatic* glucogenesis (Fig. 48-6).

HYPOTHYROIDISM. A few patients with hypothyroidism have hypoglycemia upon fasting. Since these patients, whether they have primary or secondary hypothyroidism, tend to have decreased activity of the pituitary and the adrenals, hypoglycemic reactions may be on the basis of these gland deficiencies. However, there is a possibility that the hypoactivity of liver may be out of proportion to that of other tissues and thereby permit a deficiency in hepatic glucogenesis.

HYPOGLUCAGONEMIA (?). There is no proof that a deficiency in glucagon permits hypoglycemia to develop even though this hormone has a powerful hyperglycemic action. Two of the children with hypoglycemia described by McQuarrie (discussed later) were reported to have a deficiency in α-cells, but this defect was not found in others. Animals with total pancreatectomy may experience hypoglycemia with smaller insulin dosage than alloxanized animals. Some investigators have attributed this to glucagon deficiency but others have attributed the difference to nutritional disturbances. Selective partial pancreatectomy in dogs, leaving only the uncinate process (contains no α-cells), was not associated with manifestations of glucagon deficiency. Moreover, a glucagon-deficiency syndrome has not been produced with α-cell cytotoxins (Chap. 5).

HYPOEPINEPHRINEMIA (?). A clinical hypoglycemic syndrome produced by hypoepinephrinemia is not definitely known to occur. With most adrenal operations on patients, the adrenal medullae and cortices are both removed. In demedullated animals given tolbutamide or certain other hypoglycemic agents, the amount of hypoglycemia produced is much greater than in intact animals. However, the amount of hypoglycemia produced by insulin is not influenced very much by demedullation.

HYPERSECRETION OF INSULIN

Excess insulin can, of course, cause hypoglycemia of varying degrees, including fatal. The excess of the hormone may be incited through different mechanisms (Table 48-3). This group is the most important of all. Harris, Whipple, Rynearson, and Conn are among the authors who have helped delineate this group of patients.

Neoplasms of β-cells

PATHOLOGY. Presumably more than half of the pancreatic islet cell adenomas are nonfunctional. The functional β-cell adenomas secrete excessive quantities of insulin. In the review of 398 cases by Howard, *et al.*, 9 per cent were malignant, 12 per cent questionably malignant, and 78 per cent benign. In about 12 per cent of the benign group there were multiple adenomas. The tumor occurred with equal frequency throughout the pancreas; in rare instances it was extrapancreatic, occurring in the wall of the duodenum, posterior to the pancreas, the splenic hilum, or elsewhere. The diameter varied from microscopic size to more than 10 cm., but in more than 75 per cent of the cases it was less than 3 cm. The largest number of metastases was found in the liver and regional lymph nodes.

CLINICAL MANIFESTATIONS. Although insulinomas may occur at any age they are commonest between 35 and 55. They are rare below the age of 18, no malignant insulinomas have been reported in subjects younger than 18. The sexes are involved with equal frequency. Breidahl, *et al.*, found a family history of diabetes in 24 per cent of patients with insulinoma. In patients with diabetes, 25 per cent had a family history of diabetes, but only about 4 per cent of nondiabetics have this history.

TABLE 48-4. SIGNS AND SYMPTOMS EXHIBITED BY 193 PATIENTS WITH INSULINOMAS*

Sign or symptom	*Percentage*	*Sign or symptom*	*Percentage*
Loss of consciousness	58	Noisy behavior	20
Confusional state	54	Headache	20
Weakness and fatigue	41	Tremor	18
Deep coma	40	Hunger	14
Sweating	36	Positive Babinski	13
Drowsiness and stupor	35	Paresthesias	13
Lightheadedness	30	Irritability	11
Visual disturbances	30	Transient hemiplegia	10
Amnesia	28	Abdominal pain	8
Clonic convulsions	24	Palpitation	3

* After D. L. Crain, Jr., and G. W. Thorn, *Medicine* 28:427, 1949.

The more common symptoms in 193 cases reviewed by Crain and Thorn are listed in Table 48-4. Most of the patients are not correctly diagnosed until months or years have elapsed. Many are followed by psychiatrists or neurologists because of various patterns of psychobiologic disintegration, epilepsy, or other nervous system disturbance.

The most important point in diagnosis is to be alert to this possibility. The nature of attacks exhibited by patients varies, but each subject tends to have a consistent pattern of his own. The symptomatology is most likely to appear before breakfast. Amelioration of symptoms usually occurs within a few minutes after food ingestion, but hours may be required when there has been a severe and prolonged attack. Contrary to popular belief, hunger is not a common symptom (14 per cent). Loss of consciousness occurred in 58 per cent of Crain's series. Coma developed in about 57 of 91 patients studied by Breidahl, *et al.;*

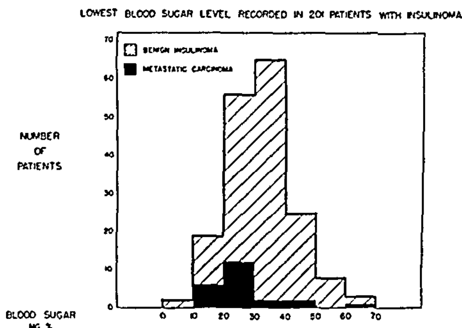


FIG. 48-10. In most patients with insulinoma a hypoglycemia can be demonstrated at one time or another. (After Crain, E. L., Jr., and Thorn, G. W.: *Medicine* 28:427, 1949.)

convulsions occurred in 37. The symptomatology with malignant insulinoma is similar to that with benign, except that it is somewhat more severe with the malignant. Crain found that deep coma developed in 95 per cent of subjects with metastatic lesions, compared with 33 per cent in the benign group. Establishment of Whipple's triad is important in the diagnosis of insulinoma: (1) history of symptoms developing with fasting and/or exercise, (2) blood sugar during attack below 50 mg. per 100 ml., and (3) prompt relief of attack by the administration of glucose.

LABORATORY TESTS. The fasting blood sugar in most patients falls below 55 mg. per 100 ml. (40 mg. per 100 ml. using true blood sugar

method) at some time (Fig. 48-10). Howard, *et al.*, found the average minimal blood sugar to be 32 In some instances repeated tests are necessary to demonstrate hypoglycemia. The glucose tolerance test has yielded results varying from flat to diabetic curves. This is not surprising when one considers the results in Figure 48-5B and that there often seem to be periodic releases of insulin from the tumor into the blood stream. The more common type of curve with a glucose tolerance test consists of a low initial level, a small glucose absorptive rise, and a low level 2 to 4 hours after the administration of glucose (Fig. 48-9). As emphasized by Conn, insulin secretion from an insulinoma apparently suppresses the activity of the normal β-cells. This function, however, can be restored by a few days of high carbohydrate feeding; the typical glucose tolerance curve can then be obtained. On the other hand, in hepatogenic hypoglycemia, the plateau curve cannot be depressed by high carbohydrate feeding.

The insulin tolerance test has not been of much assistance.

The best test consists of a 72-hour fast, which should be conducted under close supervision in a hospital. The patient is given only water, tea, and coffee without cream or sugar A blood specimen is drawn for a sugar determination upon the appearance of hypoglycemic manifestations. Breidahl found that only 1 of 73 patients with insulinoma withstood fasting without hypoglycemia for longer than 48 hours; the one exception developed hypoglycemia at 53 hours. Three patients with tumor withstood food deprivation for 48 hours during the original fast but subsequently developed symptoms in a shorter interval. One or more 72-hour fast is necessary to exclude tumor. Moreover, exercise should be combined with fasting, particularly when the latter alone does not produce hypoglycemia in a patient whose clinical manifestations suggest an insulinoma. The exercise can be given by treadmill, stationary bicycle, or other methods. Whereas fasting promotes hypoglycemia in patients with liver disease and, indeed, in many other types of hypoglycemosis, it does not tend to do so in those with functional hypoglycemia.

TREATMENT. Experience has amply demonstrated that in most instances of benign insulinoma, no therapy other than extirpation has proved satisfactory. Even subtotal pancreatectomy has not permanently eliminated the hypoglycemic syndrome when it has failed to remove the neoplasm. Since the lesion often is only a few millimeters in diameter, may exist deep in the parenchyma of the pancreas, and may not exhibit physical characteristics differing significantly from the surrounding tissue, it may be very difficult to find, but infinite persistence in search must prevail when the findings suggest the presence of a neo-

plasm because major consequences, including death, may be the alternative. However, preceding the surgical exploration in searching for the tumor, the work-up of the patient should be such as to give strong indication of the existence of an insulinoma.

Two hours before the operation the patient should begin receiving an intravenous infusion of 10 per cent glucose, which should be continued for at least 8 hours. He should also receive 100 mg. of cortisone acetate intramuscularly 2 hours preceding the operation and 100 mg. of hydrocortisone, in water-soluble form, in the glucose infusion. The glucose and the hydrocortisone should help avoid hypoglycemia, hyperpyrexia, and shock. Crain recommended that potassium and phosphorus be added to the infusion mixture.

Because the neoplasm may be difficult to find and because in more than 10 per cent of the cases multiple lesions are found, the pancreas must be mobilized for complete examination. Moreover, ectopic lesions, found in 3 per cent of cases, must be sought. Thus the peritoneum must be divided around the duodenal curve and along the superior and inferior surface of the entire pancreas. Confirmation of the anatomic diagnosis should be sought from a pathologist immediately following removal of a supposed neoplasm. Neoplasms that are readily identified are enucleated without including much surrounding tissue. In cases where no neoplasm is found, the procedure reported by Porter seems justifiable. All pancreas to the left of the superior mesenteric vessels is removed. If no tumor is found by careful sectioning by a pathologist, most of the head of the pancreas is removed, leaving enough gland to make duodenal and bile duct resection unnecessary. If no lesion is found, a radical pancreaticoduodenectomy is performed, leaving no pancreatic tissue. The experience of many clinics of finding it necessary to re-explore the pancreas until the neoplasm is removed and the greater difficulties encountered in repeated operations justify this radical and persistent search at the initial operation. However, it must be re-emphasized that the preliminary work-up of the patient should have strongly suggested the presence of an insulinoma. Porter states, "with the one exception of the patient with persistent symptoms who committed suicide before a further operation could be performed, we have had to re-explore every adult patient in whom no tumor was found originally and in whom no other cause for hypoglycemia was evident. There has been temporary alleviation of symptoms in some cases of partial pancreatectomy when no tumor was found but, sooner or later, these, in our experience, have come to exploration even though the pathologist sometimes had made a comfortable diagnosis of hyperplasia on the original tissue resected."

In children, insulinomas are much rarer than in adults. In those not responding satisfactorily to ACTH and diet therapy (see McQuarrie syndrome), the body and tail of the pancreas are resected. Experience demonstrates that in children in contrast to adults, the symptoms may be appropriately alleviated whether or not hyperplasia of the islets is found.

No effort is usually made to extirpate metastatic lesions, because they usually prove fatal, irrespective of the nature of therapy. However, some relief of symptoms is afforded by frequent feeding of a high protein, high calorie diet and with corticosteroid therapy. Repeated glucagon administration has also been reported to be of some advantage. Alloxan therapy may be tried but has not proved very satisfactory; the normal islet tissue is damaged more than the insulinoma.

The immediate postoperative mortality has been found to vary from approximately 5 to 15 per cent, depending particularly upon the extensiveness of the operation and upon appropriate therapy with corticosteroids, glucose, antibiotics, etc.

Shock and/or severe hyperthermia has been a troublesome immediate postoperative complication in some cases. However, ACTH or corticosteroid therapy help avoid these. Infection and fistula formation also offer problems. Many patients have diabetes appearing immediately postoperatively but, except in cases with total pancreatectomy, this usually disappears within 2 weeks. The diabetes must be controlled in accordance with the usual principles applied in treating diabetes and careful attention must be paid to the general nutritional state. Some pancreatic digestive enzyme preparation should be administered.

POSTOPERATIVE RESULTS AND PROBLEMS. When all the neoplastic tissue has been removed, the results are usually dramatic. Many major psychiatric and neurologic manifestations have been found to disappear. On the other hand, some will have already developed permanent brain damage and may continue with dementia, parergastic reactions, hemiplegia, or motor neuronitis (with muscular atrophy).

Diffuse Hyperplasia

Diffuse hyperplasia of the islets as a cause of hypoglycemia has not been clearly delineated as a separate entity. It has been observed in a few patients with neurogenic hyperinsulinism and in some with insulinoma. It also is noted in animals following the administration of somatotropin, ACTH, corticosteroid, thyroxin, and estrogen.

Therapy consists of frequent feedings of a high protein–low carbohydrate diet. In some instances, particularly children, some benefit may be derived from partial pancreatectomy.

Posthyperglycemia Neurogenic (Right Vagus)

This is the commonest type of spontaneous hypoglycemia. It apparently is due to hyperstimulation of the β-cells by the right vagus nerve. As discussed earlier, hyperglycemia stimulates the right vagus and it increases insulin secretion. With the entity under consideration a hyperresponse is obtained. It has been described as functional hypoglycemia, functional hyperinsulinism, nervous hypoglycemia, hypoglycemic fatigue, and reactive hypoglycemia. Patients with this type of hypoglycemia tend to be emotionally unstable, dynamic, tense, anxious, and very conscientious. They have manifestations of a hyperactive autonomic nervous system, including hypermotility of the gastrointestines and increased gastric acidity. Psychosomatic problems are common.

Hypoglycemia does not tend to occur during fasting. It is induced by eating, particularly eating carbohydrates. Hypoglycemic symptoms appear 2 to 4 hours after a meal. The symptoms are those of hyperepinephrinemia (weakness, perspiration, anxiety, nervousness, and tachycardia) and of cerebral anoxia (mental confusion, visual disturbances, faintness). Hunger may be present. There is no loss of consciousness, convulsions, or other manifestations of severe hypoglycemia; all the hypoglycemic manifestations are reversible. The intensity of the reactions, unlike those with insulinoma, are not necessarily progressive; they vary considerably with the emotional status of the patient.

Conn states that "the oral glucose tolerance is pathognomonic [Fig. 48-11], providing that standard dietary preparation is employed." The fasting blood sugar and the hyperglycemic peak are normal, but between *2 to 4 hours after glucose administration* the blood sugar level descends below 40 mg. per 100 ml. and hypoglycemic symptoms appear.

Although the glucose curve shown in Figure 48-11 is one of the most characteristic, this pattern must not be expected in all patients giving a history suggestive of posthyperglycemia neurogenic hypoglycemia. The subjects vary periodically in their emotional reactions, eating habits, etc., and consequently, different types of curves are observed in different subjects and even in the same subject. It may be necessary to perform several glucose tolerance tests in order to demonstrate hypoglycemia. Moreover, for adequate assurance of the diagnosis it is desirable to elicit not only hypoglycemia but also hypoglycemic symptoms. There often is not a good correlation of the blood sugar and the symptomatology because the former is not a direct indicator of glucose utilization by the brain.

Fabrykant studied glucose tolerance curves and related symptomatology in 50 patients with "neurogenic functional hypoglycemia." After

a 3-day to 5-day preparatory high carbohydrate diet the test was conducted with measurements of true blood glucose in venous and capillary blood for hourly or shorter intervals up to 5 or 6 hours. He concluded that in 62 per cent there were clinical entities which, within themselves, could account for hypoglycemia and/or the symptomatology—for example, hypoadrenocorticism.

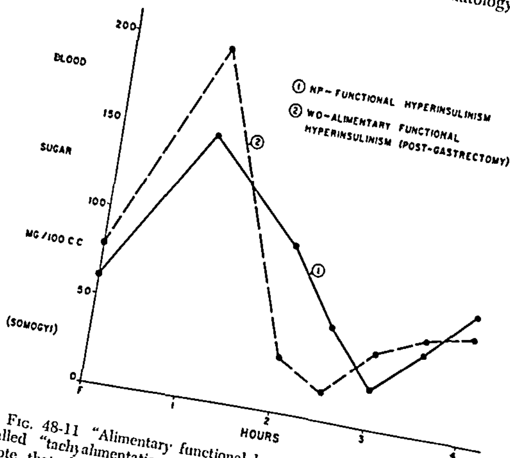


FIG. 48-11 "Alimentary functional hyperinsulinism" is what I have called "tachyalimentation (posthyperhyperglycemia) hypoglycemia." Note that the hyperglycemic peak is greater and the nadir occurs earlier with the tachyalimentation type. (After Conn, J. W., and Seltzer, H. S. *Am. J. Med* 19.460, 1955.)

Therapy is directed toward correction of factors producing emotional instability, along with frequent feedings of a high protein, high fat and low carbohydrate diet. Anticholinergic drugs sometimes have an additional value.

Tachyalimentation (Posthyperglycemia)

Some patients who have been subjected to gastric resection or gastroenterostomy have hypoglycemic attacks that appear postprandially. The

glucose tolerance test is characterized by a normal fasting level, a hypernormal glucose absorptive peak, and a hypoglycemic level approximately 2 hours after the administration of glucose (Fig. 48-11). The symptomatology is like that discussed in the immediately preceding section. Apparently the gastrointestinal surgery promotes a rapid passage of glucose into the small intestines, thereby accelerating glucose absorption. A similar phenomenon is observed in normal persons to whom glucose is administered by intraduodenal tube. The marked hyperglycemia is visualized as stimulating excess insulin secretion that eventually leads to hypoglycemia.

A concept that deserves consideration is that many patients with this type of hypoglycemia have or have had a peptic ulcer. Patients with peptic ulcer, like those with posthyperglycemia neurogenic hypoglycemia, are prone to have vagotonia. Moreover, Berry, in discussing reports of 10 clinical studies in patients with peptic ulcer subjected to a glucose tolerance test, points out that an average of 35 per cent (probably falsely high because of normal standard selected) were found to have hypoglycemia toward the end of the test. Many of these ulcer patients experienced clinical hypoglycemic symptoms after a glucose tolerance test or 1.5 to 3 hours after a meal. The following pertinent observations have been published relative to peptic ulcer patients: (1) 25 per cent have hyperplasia of the pancreatic islets; (2) there is an increased sensitivity to insulin; (3) peptic ulcer is only 25 per cent as common in diabetics as nondiabetics (no case of peptic ulcer was observed in 4,103 diabetics studied before the introduction of insulin) Experimentally hypoglycemia has been found to be conducive to peptic ulcer, whereas glucose administration makes certain ulcerogenic agents less effective.

In the light of the foregoing reports, consideration should be given to the possibility that certain patients with vagotonia develop posthyperglycemia neurogenic hypoglycemia and also peptic ulcer. Then after certain gastric operations these subjects absorb glucose more rapidly, producing hyperhyperglycemia, which leads to an increased rate of insulin secretion, accounting for the difference in the glucose tolerance test illustrated in Figure 48-11. This type of hypoglycemia could be visualized as a posthyperhyperglycemia, neurogenic type. Zollinger has described a clinical entity consisting of [illegible] [illegible]cretion, hyperacidity, and atypical peptic ulceration associated wi[illegible] [illegible]lin-producing islet cell tumors of the [illegible] The question [illegible] to whether these ulcer patients are p[illegible] [illegible] *hypo*[illegible] [illegible] *with an increased* need for glucag[illegible] a tumor of [illegible]oducing [illegible]lls.

Therapy [illegible] [illegible] fee[illegible] [illegible] high fat (except in [illegible] [illegible]ohydrate [illegible] [illegible] be

given as needed. The dumping syndrome must be differentiated from the hypoglycemic syndrome. The former appears within 30 minutes after good ingestion and disappears within an hour. Postprandial hyperosmolar jejunal contents draw fluid rapidly into the lumen, causing epigastric fullness, nausea, sweating, weakness, and palpitation; hypoglycemia is not present during the symptoms of the dumping syndrome.

INSULIN HYPOANTAGONISM

In earlier sections it was related that the pituitary and the adrenals antagonized the action of insulin. With decreased or absent function of these glands insulin action is much stronger and may be associated with severe hypoglycemia.

Therapy consists of frequent feedings of a high protein, low carbohydrate diet and of the administration of corticosteroid.

PHARMACOGENIC OR TOXIGENIC COMPOUNDS

EXCESS INSULIN

Administration of exogenous insulin is the commonest cause of hypoglycemia and produces more permanent damage than all the other causes combined.

FACTITIOUS INSULIN. In some instances the subject is not diabetic, but injects himself with insulin (factitious) either for suicidal intent or because of a strange psychologic complex. In such situations a major detective *effort may be required; sometimes the insulin ampoule may* be hidden in the rectum or some other such obscure and unsuspected spot. If such episodes have occurred for several weeks, insulin antibodies in the subject's plasma should be demonstrable.

MISTAKES IN INSULIN DOSAGE

Frequency

Hypoglycemic reactions in diabetics requiring insulin are common. They may be severe and associated with permanent damage to the nervous system or may cause death. In the author's opinion the damages are more common and more serious than is generally recognized. Greenblatt reported that 51 per cent of diabetics with frequent severe insulin reactions had abnormal electroencephalograms. In studying 203 diabetics treated with insulin, Goodman found that 38 per cent had experienced insulinogenic hypoglycemic reactions. The more severe and unstable the diabetes the more frequent and severe were the reactions; 55 per cent of the severe diabetics had had insulin reactions. Protamine zinc insulin and its various mixtures were the most common offenders, accounting for

81 per cent of the reactions. With regular or globin insulin the reactions were much less frequent and less severe. Reactions to regular insulin are most apt to appear after 3 to 4 hours, after 6 to 8 hours with NPH or globin insulin, and after 12 to 24 hours with protamine zinc insulin.

Hypoglycemic Symptoms

The more rapid the development of hypoglycemia, the more prominent are the symptoms. *The reactions to regular insulin are more* apparent to the patients because the symptoms appear faster and more dramatically, the sympathetic manifestations predominating. The reactions to protamine zinc insulin appear much more insidiously and are related chiefly to cerebral anoxia. Whereas manifestations of hyperepinephrinemia are also present, they are less apparent to the patient because of the associated mental cloudiness, moreover, many of these reactions appear during sleep during the early morning hours. Patients differ considerably in the type and intensity of their hypoglycemic symptoms, but the pattern tends to be repeated in the same subject. In the early phases there tends to be mental and physical retardation. There is weakness, fatigue, nervousness, anxiety, nausea, headache, mental confusion, drowsiness, and pallor. There also may be hunger, irritability, and restlessness, transient diplopia, vertigo, ataxia, and tremor. Speech and thinking are difficult. After about 15 to 30 minutes there may be more excitement, restlessness, paraesthesias, and hypalgesias, but also an increase in confusion and perspiration; delirium and a great variety of parergastic and thymergastic patterns may be revealed. Finally, coma with or without convulsions may appear along with neuropathic signs, such as positive Babinski reflexes, nystagmus, aphasia, Parkinsonism, palsies, etc.

Adlersberg has emphasized a number of important medicolegal and social aspects of hypoglycemia. Very bizarre reactions, quite different from the usual ones of the patient, may appear. For example, there may be highly abusive acts committed by one who is ordinarily most kind and considerate, exhibitionism, sexual perversity, negativism, etc. Traffic accidents, assaults and various other types of misconduct may occur. An effort must be made to protect the patient and society.

Illustrative Cases

The following case reports illustrate some major problems resulting from hypoglycemia.

Juvenile diabetic with posthypoglycemic imbecility. H. C, a female aged 21, from a small town, was hospitalized with diabetes, marked mental de-

ficiency, and illegitimate pregnancy. She had the onset of diabetes at age 7. It was fairly well controlled until age 13, when it became very unstable. Up to 200 units of protamine zinc insulin were administered daily but there were many convulsions. Many times she had glycosuria and drowsiness for which increased insulin was given, resulting in more drowsiness and disorientation. Blood sugar was frequently found to be very low. Until a couple of years after the onset of the severe insulin reactions she was intelligent, but subsequently exhibited such marked mental deficiency that she was institutionalized for custodial care. She became very slow in all mental and physical activities and her memory became very poor. She developed aphasia and hemiparesis. Careful analysis of her case indicated a good correlation of the severe hypoglycemic reactions with the psychic and neurologic disorders.

Comment. This problem is a common one faced in unstable diabetics. The patient's mother frequently called the physician and reported that her daughter was drowsy and confused but that there was significant glycosuria. Consequently, additional insulin injection was recommended, intensifying the hypoglycemic reaction. The sugar had collected in the bladder before the hypoglycemia developed. To avoid this common error, the urine should be collected for a short interval and examined.

Severe brain damage and death from a hypoglycemic reaction. J. H., a male aged 45 with a ten-year history of diabetes, was hospitalized in coma. He ordinarily received 80 units daily of insulin. He previously had had insulin reactions and convulsions with one of them. He lived alone and was last seen well 57 hours before death, at which time he was in his room drinking whisky in celebration of his birthday. When seen 33 hours antemortem, he was lying in bed in deep slumber, breathing hard. When next seen, 9 hours antemortem, he was in deep coma and was brought to the hospital. The major observations were: pulse rate of 140, respiratory rate of 40, shallow respiration, temperature of 102° F., blood pressure of 120/60, deep coma, flaccid extremities, deviation of head and eyes to the right, absent deep tendon reflexes, and no response to pain. The urine showed sugar (1+) and acetone (1+) The blood sugar was 505 mg. per 100 ml. (this was checked; no food had been given). The blood CO_2 combining power was 18 mEq. and the serum acetone was 3+. The hematocrit was 53. Saline and 5 per cent glucose were infused intravenously, but the patient showed no improvement, and death occurred in 3 hours Pulmonary congestion and pulmonary edema had developed Autopsy showed extensive brain damage attributable to hypoglycemia (Fig 48-12).

Comment. The setting for hypoglycemia in this case, as in many others, was acute alcoholism with associated changes in diet, exercise, emotions, circulation, insulin injections, etc. Moreover, the alcoholic intoxication misled his friend with respect to insulin reaction It is not known when he received his last insulin injection, but it is most likely that none was received for at least 33 hours antemortem. Thus, by the time he was hospitalized he no longer had hypoglycemia but, instead, marked hyperglycemia and also keto-

nuria, and the brain damage was already so severe that death was inevitable. However, in cases seen earlier, such is not the case and it must be recognized that hyperglycemia and ketonuria may soon follow hypoglycemia, because of decreased glucose utilization and decreased lipogenesis.

Death from hypoglycemia, following cessation of ACTH therapy. E. A., a housewife aged 68, received ACTH therapy for pemphigus, 20 mg. per day

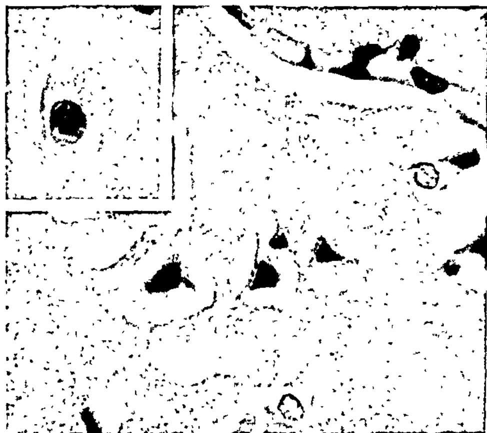


Fig. 48-12. Cerebral cortex. Cytodegenerative changes of neurons in severe hypoglycemia. Three shrunken neurons with pyknotic nuclei and enlarged perineuronal spaces. (H&E × 640.) (*Inset*) Normal neuron for comparison. (H&E × 640.) (Courtesy of Dr. Raymond Ham.)

for 2 weeks and then 75 to 100 mg. per day, given continuously for 3 weeks intravenously. Although the patient specifically denied any family history of diabetes and the urine was free of sugar preceding ACTH and again 12 days after initiation of this therapy, the urine repeatedly contained 5 to 7 per cent sugar 3 weeks after starting treatment with ACTH. Insulin therapy was instituted, but almost all premeal and prebedtime urine specimens contained sugar in spite of 40 units of protamine zinc insulin and approximately 40 units

of regular insulin daily. After the administration of ACTH for 5 weeks the skin lesions were almost entirely gone, but the patient developed mental depression. The ACTH infusions were stopped at 3 P.M., 24 hours before death. It was planned to give ACTH in daily decrements, permitting cessation of therapy within a week. Since the possibility of insulin reactions was firmly borne in mind, it was planned to decrease insulin dosage accordingly. The urine yielded a 4+ sugar reaction at 6:00 P.M. and 3+ at 9:00 P.M.; regular insulin was given in doses of 20 units and 15 units respectively. At 3:00 A.M. the patient seemed to the nurse to be satisfactory, but at 5:30 A.M. she was comatose, flaccid, and had rapid, shallow respirations. The blood sugar was 17 mg. per 100 ml. and there was no glycosuria. In spite of large doses of 50 per cent glucose and ACTH intravenously, the patient died at 2:30 P.M. Autopsy demonstrated cerebral damage consistent with profound hypoglycemia.

Comment. The case illustrates the enormous importance of the balance of ACTH and insulin activity. Without previously evident diabetes, ACTH precipitated severe frank diabetes and then on cessation (too rapidl) of ACTH, insulin induced a fatal hypoglycemia. Only 8 hours before the severe hypoglycemia was recognized and therapy for it was instituted, the patient had marked glycosuria and needed insulin. Despite vigorous treatment for hypoglycemia, death resulted because there had already developed too many irreversible brain changes.

Hemiplegia produced by hypoglycemia and alleviated by glucose administration. F. S., a salesman aged 37, had had diabetes for 8 years. He usually took 35 units of protamine zinc insulin and 5 units of regular insulin, but he lived alone and was not very attentive to diet or urinary examinations. One morning about a week before hospitalization, he developed syncope upon arising from bed. He thought he remained unconscious for a few minutes. He then observed typical left hemiparesis, which lasted for a few seconds. On the evening preceding hospitalization he took 30 units of protamine zinc insulin and 5 units of regular insulin. He awakened at 6:00 A.M. on the floor, soiled with feces, and noted that the left side of his body was numb and paralyzed. On examination he exhibited a typical hemiplegia. The blood sugar was 31 mg. per 100 ml. He was given 25 gm. of glucose intravenously and within 3 minutes there were only minimal neurologic abnormalities and none after an hour. The electroencephalogram was normal before and after recovery from the hemiplegia.

Comment. Transient hemiplegia not uncommonly occurs with hypoglycemia. It becomes permanent when therapy is delayed unduly.

Considerations in the Prevention and Treatment of Hypoglycemia

It is clearly apparent from the previous discussion that insulin is a powerful compound and can cause death in a few hours or cause permanent damage to the nervous system or to the heart. Moreover,

severe reactions are common. Prevention of the insulin reactions, particularly the severe ones, is the most important goal and in considering this objective, many phases of the pathogenesis and course of diabetes must be considered. Most of the difficult problems are presented by the unstable ("brittle") diabetics who, within a few hours, can change from a state of marked hyperglycemia and ketonemia to severe hypoglycemia and vice versa. Relatively slight changes in diet, exercise, insulin dosage, emotional reactions, infection, and various other factors can affect tremendously the degree of control of the diabetes. Stability in all phases of living is important in this group. Most of the subjects who develop permanent damage are ones who have exhibited an unstable control over a long interval. These patients are supposedly markedly deficient in their supply of effective insulin and have to depend chiefly upon an exogenous supply. Whereas the amount of endogenous insulin entering the blood decreases when hypoglycemia develops, the amount of exogenous insulin absorbed from the injection site continues unabated. The unstable diabetic often has a subnormal supply of carbohydrate stored in liver and muscle and consequently cannot combat hypoglycemia as well as normals. With the outpouring of corticosteroids and epinephrine the hypoglycemia may spontaneously disappear, but they antagonize glucose utilization which, when inadequate, damages the tissue, rather than the hypoglycemia per se. In the author's opinion, many of the unstable diabetics do not receive enough insulin or food. Experience with Cushing's disease has demonstrated that marked glycosuria over many years is not often associated with diabetic acidosis, retinopathy, or intercapillary glomerular sclerosis. Apparently, patients with Cushing's disease secrete enough insulin to avoid these complications. It seems that it would be desirable, by increasing the diet and insulin of the unstable diabetics, to obtain better stores of carbohydrate, fat, and protein and thereby decrease many of the complications, including hypoglycemia. It is better to have some glycosuria, particularly when an abundance of insulin is given, than to produce significant hypoglycemic attacks. It is especially desirable to avoid hypoglycemia in the elderly, because this group is prone to develop damage to the brain and/or the heart. Their circulation may already be poor and, if so, damages from hypoglycemia are more likely. Indeed, various types of previous injuries to the brain seem to intensify hypoglycemic manifestations; previous hypoglycemic damages also seem to encourage subsequent ones.

Another factor in preventing hypoglycemia consists of unusual alertness to decreased insulin requirements following disappearance of infections, unusual strenuous exercise, various stresses, and insulin re-

sistance. Also should be noted decreased activity of the liver or kidney and decreased supply of adrenal, pituitary, or thyroid hormones.

A crucial point in the management of hypoglycemia is its early recognition and prompt therapy. As indicated earlier, a patient may have a severe hypoglycemic reaction and yet have glycosuria, the sugar having entered the bladder before the development of hypoglycemia. Moreover, the hypoglycemia may be followed by the spontaneous development of hyperglycemia and ketonemia. Despite the hyperglycemia, there may be a marked decrease of glucose utilization by the brain; administration of 95 per cent oxygen and of intravenous glucose is of advantage in some cases.

OTHER AGENTS

A vast number of compounds can cause hypoglycemia. Included are: biguanides, guanidines, sulfonylureas, hypoglycin, amino acids, somatotropin, prolactin, alloxan (early), hydrazine, Antistine, Antergan, Neoantergan, Pyribenzamine, mesoxalic acid, nicotinic acid, salicylate, pyrazinoic acid, amphenone, and many others. In many instances the mechanism of production of the hypoglycemia is not known. Some of these compounds have been found to increase the secretion of insulin, decrease insulin degradation, decrease hepatic glucogenesis, decrease gluconeogenesis, or to increase glucose uptake in association with a state of anaerobiosis that has been produced. Some of these compounds are taken as medicines and some are taken by mistake in the diet. Thus, this must be borne in mind in all unexplained hypoglycemias.

A detailed discussion of some of these hypoglycemic compounds is presented in Chapter 35.

IDIOPATHIC HYPOGLYCEMIAS

Infantile (McQuarrie Syndrome)

McQuarrie has studied a group of infants with familial idiopathic hypoglycemia. This entity constituted more than half of all the hypoglycemias seen on McQuarrie's pediatric service; it was much more common than diabetes. The hypoglycemic manifestations almost always begin appearing before the age of 2; the disorder is rarely seen after the age of 8. It is more common in males. The etiology is not known. Evidence suggests that it is not due to deficiency of the pituitary, thyroid, adrenals, or liver. Two patients were found to have an absence of α-cells in the pancreas, suggesting that glucagon deficiency might contribute to the hypoglycemia. However, subsequent cases were found *not* to have α-cell deficiency. Dietary abnormalities do not seem to play an

etiologic role. It could be related to decreased insulin degradation, but specific studies along this line have not been reported. No physical stigmata are observed; weight and body measurements are normal.

The hypoglycemic symptoms are like those produced by other causes. There may be convulsions and coma. Moreover, permanent brain damage sometimes occurs. The condition varies in severity from time to time. The hypoglycemia and its symptoms are especially apt to appear with fasting. With a glucose tolerance test, subnormal values are obtained for the fasting level, the hyperglycemic peak and in the 1.5-hour to 3-hour specimens. Thus the results are similar to other conditions associated with hyperinsulinism, for example, insulinoma or excess insulin injection.

There are other instances of hypoglycemia appearing in infants which apparently are of a different type. Babies, during the first two weeks of life, may have hypoglycemia, but it is asymptomatic and not persistent. Sometimes a baby born of a diabetic mother develops fasting hypoglycemia; this is a transient disorder that presumably results from hyperplasia of β-cells induced by hyperglycemia. During infancy some children have hypoglycemia postprandially, apparently resulting from excessive insulin liberation (Staub-Traugott phenomenon); in this instance the fasting blood sugar is normal and the phenomenon subsides upon restricting carbohydrate intake. Sometimes in children there is hypoglycemia produced by a deficiency of hydrocortisone as in the congenital adrenal hyperplasia. In addition, there may be a large number of other causes such as galactosemia, Von Gierke's disease, etc.

McQuarrie has reported that all cases of idiopathic infantile hypoglycemia have responded satisfactorily to ACTH therapy; the response to corticosteroids was definitely less good. He recommended the administration of 4 mg. of ACTH per kilogram of body weight, given intramuscularly in 4 doses (every 6 hours) daily for 4 days and then for the following week one fourth of this dosage, given in 2 doses daily of ACTH gel. When the fasting blood sugar remains normal, the morning dose is omitted during the succeeding weeks. The dosage is progressively decreased until it is finally eliminated. The guiding criteria are the maintenance of a symptom-free state and fasting blood sugar levels above 40 mg. per 100 ml. The dosage of ACTH must be individually regulated in each patient.

It must be borne in mind that whereas benefits from ACTH have been very good in these cases, some benefit from it has also been observed in patients with pituitary insufficiency, congenital adrenal hyperplasia, glycogen storage disease, insulinoma, and other conditions. Insulinoma in this age group is very rare.

Early Diabetes

Seltzer, *et al.*, have emphasized that many patients in the early phase of diabetes may have attacks of spontaneous hypoglycemia appearing particularly 3 to 5 hours after meals. Indeed, this is one of the commonest types of spontaneous hypoglycemia. The authors state that such attacks "represent the earliest clinical manifestation of diabetes in many

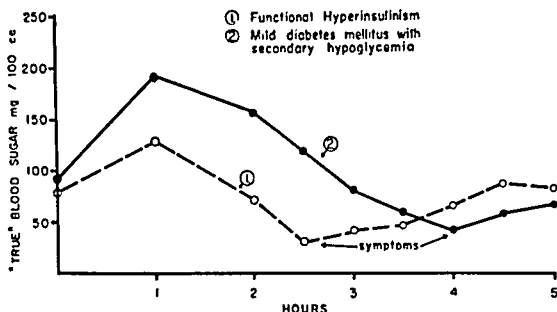


Fig. 48-13. With "functional hypoglycemia" the glucose tolerance test shows the fasting blood sugar level to be usually normal with a hyperglycemic peak below 140 mg. per 100 ml. and a hypoglycemia (usually with symptoms) between 2 and 4 hours. In mild diabetics with spontaneous hypoglycemia, the fasting blood sugar is normal or slightly elevated, the hyperglycemic peak above 160, hyperglycemia at 2 hours and hypoglycemia, with symptoms, between 3 and 5 hours. (After Seltzer, H. S., Fajans, S. S., and Conn, J. W.: *Diabetes* 5:437, 1956.)

cases." They found 110 patients whose glucose tolerance tests were characterized by (Fig. 48-13) (1) a normal or slightly elevated fasting blood sugar, (2) hyperglycemia that is prolonged for the first 2 hours or more, and (3) a sudden fall to hypoglycemic levels between the third and fifth hours. The criteria used were: (1) a one-hour blood sugar of 160 mg. per 100 ml. or above, (2) a two-hour blood sugar of 120 mg. or above, and (3) a blood sugar (true) of 50 mg. or below. It is important to conduct the test for an interval of 4 to 5 hours.

Sixty-nine per cent of Seltzer's patients had complained of postpran-

dial hypoglycemic symptoms and 44 per cent had a family history of diabetes. Thirty-six per cent had symptomatic hypoglycemia and a family history of diabetes.

The mechanism involved in the production of hyperglycemia and hypoglycemia is not known. Seltzer, *et al.*, suggested that there may be a delayed response in insulin secretion to hyperglycemia, but finally more insulin becomes available than is needed at the time.

Therapy consists of a reduction in caloric and carbohydrate intake in patients of normal weight. In a few such patients, small doses (10 to 15 units) of insulin, as well as carbohydrate restriction, are indicated.

SUMMARY

When hypoglycemia is severe and prolonged, permanent damage, including death, may occur. The brain is the organ affected most since it depends for energy almost entirely upon carbohydrates. When these are not supplied, there is a decrease in cerebral oxidation and cerebral changes characteristic of anoxia are observed. Cerebral A-V oxygen and glucose differences correlate with cerebral function much better than the blood sugar. Anoxia stimulates the sympathetic nervous system and depresses the somatic. The order of involvement of the brain is the reverse to the appearance of different portions phylogenetically; the functions of the cerebral cortex and cerebellum are affected first and the medulla oblongata last. Manifestations may appear in the following 5 stages: (1) somnolence, perspiration, hypotonia, and tremor; (2) loss of consciousness, primitive movements, twitches, restlessness, clonic spasms, hyperresponsiveness to pain; (3) tonic spasms, inconjugate ocular deviation, Babinski sign; (4) extensor spasms, and (5) deep coma, shallow respiration, bradycardia, miosis, loss of light reflex, hypothermia, atonia, hyporeflexia, and death.

Some of the features of hypoglycemia are experienced by patients affected by any one of a large number of diseases. Among the group necessitating differentiation are: the hyperventilation syndrome, anxiety neurosis, neurocirculatory asthenia, hysteria, parergasia, thymergasia, various syncopes (particularly emotional and carotid sinus), orthostatic hypotension, epilepsy, brain tumor, alcoholism, diabetic ketoacidosis, chemical poisons, parathyroid diseases, cerebrovascular accidents, and uremia. As emphasized by Edwards, the hyperventilation syndrome, in particular, should be differentiated. It, like some of the hypoglycemoses, is found in emotionally unstable subjects and each may be associated with faintness, weakness, palpitation, nervousness, anxiety, sweating, tachycardia, vasomotor changes, mental confusion, syncope, headache,

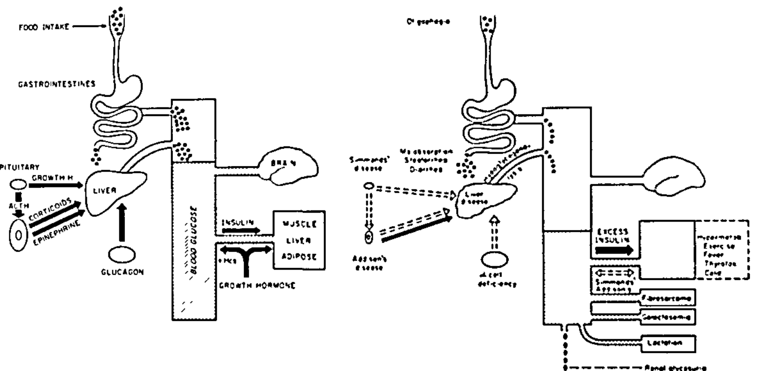


Fig. 48-14. A diagrammatic representation of some of the common factors involved in hypoglycemia. The brain suffers as the supply of glucose to the blood is decreased, whether via decreased absorption from the gastrointestines, excess elimination in milk or urine, increased utilization because of hypermetabolism, decreased glucogenesis from the liver, or by diversion of energy in various ways.

dial hypoglycemic symptoms and 44 per cent had a family history of diabetes. Thirty-six per cent had symptomatic hypoglycemia and a family history of diabetes.

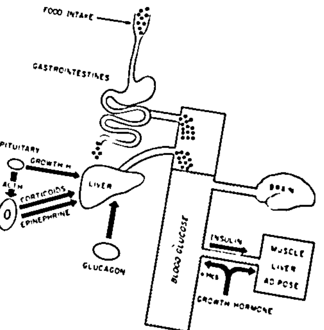
The mechanism involved in the production of hyperglycemia and hypoglycemia is not known. Seltzer, *et al.*, suggested that there may be a delayed response in insulin secretion to hyperglycemia, but finally more insulin becomes available than is needed at the time.

Therapy consists of a reduction in caloric and carbohydrate intake in patients of normal weight. In a few such patients, small doses (10 to 15 units) of insulin, as well as carbohydrate restriction, are indicated.

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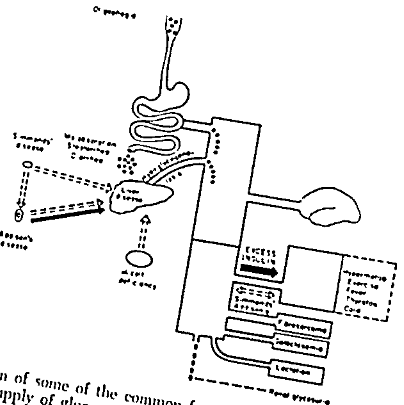


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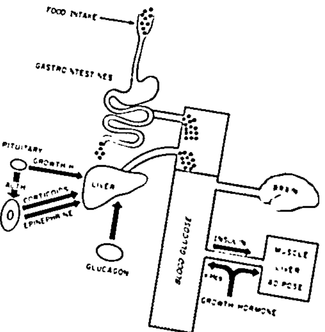
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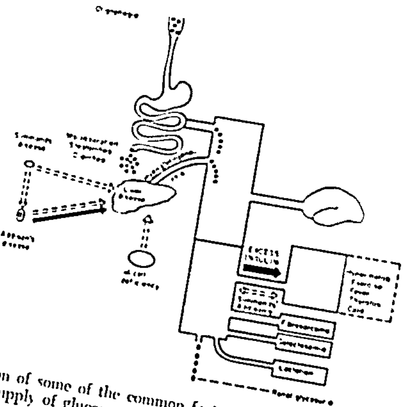


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and visual disturbance. Moreover, the two conditions occur together not uncommonly.

A large number of factors influence the blood sugar concentration. The major ones are: (1) food supply, (2) food demands, (3) food transformations and release in the body, particularly in the liver, (4) amount of insulin, (5) insulin antagonism, and (6) hypoglycemic agents.

The major point in diagnosing hypoglycemosis is frequently to entertain its possible existence and then systematically to consider it and its etiology. The amount and type of food ingested is important, as is food wastage, for example, lactation, glycosuria, and fecal loss via steatorrhea, etc. Hypoglycemia may also result from excessive glucose utilization (exercise, fever, thyrotoxicosis, or neoplasms). In this group the fasting blood sugar tends to be low. There may be varying types of curves with the glucose tolerance test. Treatment consists of more food.

Hypoglycemia may result from decreased liver function with an associated decreased glucogenesis, such as occurs with severe cirrhosis or hepatitis. The hypoglycemia is most apt to appear during fasting. The glucose tolerance test shows a low initial level and a prolonged hyperglycemia peak that slowly returns to normal. Sometimes there is a postprandial hypoglycemia. There also is a decreased hyperglycemic response to epinephrine and glucagon. Occasionally there may be a decrease in the liver enzymes concerned with glucogenesis, such as in Von Gierke's disease. The decreased liver glucogenesis may be secondary to certain hormone deficiencies: somatotropin, corticosteroids, and thyroxin, and possibly also epinephrine and glucagon.

Patients with insulinoma tend to have most of their symptoms while fasting. Indeed, the best test for diagnosis is a 72-hour fast. Particularly when this is repeated and combined with exercise, very few patients with insulinoma fail to show a significant hypoglycemia. Such patients should then be subjected to as extensive exploration as is needed to find and remove the tumor; otherwise the entire pancreas should be removed.

Glucose, fatty acids, and amino acids each stimulate, chiefly via the right vagus, increased insulin secretion. Some patients with vagotonia apparently respond excessively, producing too much insulin, and have hypoglycemia with symptoms 2 to 4 hours after glucose ingestion (posthyperglycemia neurogenic hypoglycemia). Unlike most other hypoglycemoses, fasting does not tend to cause hypoglycemia in this group. With this type, in comparison with insulinoma, the symptoms are less severe, more variable, less progressive, more responsive to diet and tend to occur in subjects who were emotionally labile even before the hypoglycemia manifestations appeared. Moreover, in this group the

symptoms almost always follow food ingestion. Therapy consists of frequent feedings of a high protein, high fat (unless obesity is present), low carbohydrate diet; anticholinergic drugs may also be given. In patients who have had gastric resection or gastroenterostomy with tachyalimentation there may be excessive elevation of the blood sugar following food, but within 1.5 to 3 hours a hypoglycemic reaction. Apparently the hyperhyperglycemia stimulates excessive insulin; vagotonia may be important in this group. Therapy is the same as in the posthyperglycemia neurogenic type.

The commonest and the most damaging of all the hypoglycemias is that produced by the administration of exogenous insulin. A large variety of neurologic and psychopathic patterns have been produced, as well as more than a few deaths. Most of these reactions occur in unstable diabetics with the use of protamine zinc insulin and its derivatives. It is important for insulin therapy not to provoke severe reactions; this may necessitate a more liberal diet and more insulin, frequent feedings, and changes in the quality of diet as well as in its daily distribution. Early recognition and treatment of the hypoglycemic reaction is important. It must be borne in mind that sugar may be found in urine obtained during a reaction if the patient has not micturated for several hours. Genuine glycosuria and ketonuria may appear within a few hours before or after hypoglycemia Finally, the level of the blood sugar does not always correlate with the intensity of the hypoglycemic symptoms. The rate of cerebral glucose *utilization* is the important factor.

There are many compounds used as drugs or occurring in the diet that may cause hypoglycemia.

A congenital and familial hypoglycemia has been described that appears in infants and disappears spontaneously in a few years. However, permanent brain damage may occur. Therapy consists of frequent feedings of a high protein, high fat diet, and ACTH therapy.

A plea is made for greater attention to the diagnosis and therapy of hypoglycemoses. In the author's opinion, we of the medical profession have usually done a poor job in this field.

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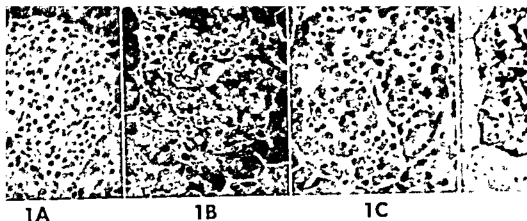
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